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October 5, 1989
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Edited by R.G.L. McCready

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Foreword

This report contains the technical papers given by BIOMINET members at the 1989 annual general meeting, which was held in Laval, Quebec, on October 5, 1989.

R.G.L. McCready
Editor

Avant-propos

Ce rapport comprend les exposés techniques présentés par les membres de BIOMINET à la réunion générale annuelle de 1989 qui s'est tenue le 5 octobre 1989 à Laval, Québec.

R.G.L. McCready
Éditeur

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Selective Concentration of Uranium from Bioleach Solutions Using Immobilized Biomass in a Continuous Pilot Plant

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Abstract

Continuous pilot-plant experiments using immobilized *Rhizopus arrhizus* biomass were conducted to remove and concentrate uranium contained at low concentrations (95 to 150 mgU/L) in Denison Mine bioleach solutions from Elliot Lake, Ontario. Biomaterial was physically and chemically characterized and used in a column 6.4 cm in diameter and 1 m tall. Twelve elements were analyzed to better understand the process. Up to a 40-fold uranium concentration was obtained. During elution, concentrations as high as 13.5 gU/L were obtained. The process was shown to be selective for uranium. Uranium uptake capacity of the biomaterial at the bottom of the column was adversely affected by competing ions, but uranium uptake capacity of the biomaterial at the top was constant after the second loading-elution cycle and for the next seven cycles.

Keywords: bioadsorption, uranium, immobilized biomass

Résumé

Des expériences pilotes continues ont été réalisées en utilisant la biomasse à *Rhizopus arrhizus* immobilisée pour extraire et concentrer l'uranium contenu en faibles concentrations (de 95 à 150 mgU/L) dans les solutions de biolixiviation de la mine Denison à Elliott Lake (Ontario). Le biomatériau a été physiquement et chimiquement caractérisé et utilisé dans une colonne de 6,4 cm de diamètre et 1 m de hauteur. Pour mieux comprendre le procédé, on a analysé 12 éléments. Une concentration d'uranium jusqu'à 40 fois supérieure a été obtenue. Durant l'élution, on a obtenu des concentrations pouvant atteindre 13,5 gU/L. Ce procédé s'est avéré sélectif pour l'uranium. La capacité d'absorption d'uranium par le biomatériau, à la base de la colonne, a été négativement influencé par les ions compétiteurs tandis qu'au sommet de la colonne, cette capacité a été constante après le deuxième cycle de chargement–élution et pendant les sept cycles suivants.

Mots–clés : bioadsorption, uranium, biomasse immobilisée

Introduction

At Elliot Lake, Ontario, Denison Mines Limited produces large volumes of biological leach solutions underground, containing 80 to 300 mgU/L. Because of the high pumping costs from the large volume of biolixiviant, leach solutions have to be concentrated underground. One method for removing and concentrating uranium is ion exchange. However, this method is not viable because of the high costs and the strong acids needed to strip uranium from the resin. It is not advisable or feasible to use strong acids underground. Also, the uranium uptake capacities of resins are very low at low uranium concentrations (Tsezos and Volesky, 1981). Thus, immobilized biomass (i.e., biomass fixed on a support such as polymers) can be used in place of ion-exchange resins. Some immobilization processes have been developed (Tsezos, 1987; Jeffers *et al.*, 1989) and particularly, immobilized *Rhizopus arrhizus* biomass has been produced at McMaster University according to a proprietary process. The fungus *R. arrhizus* was chosen because it has been shown to be effective in recovering uranium at low concentrations (Tsezos and Volesky, 1981; Tsezos and Volesky, 1982).

Most studies on uranium sorption by microorganisms have been carried out on a small laboratory scale. A few years ago, biosorption of uranium from mine process solutions by various microorganisms was assessed (Acres Davy McKee Eng. Inc., 1986; Senes Consultants Ltd., 1985; Byerley *et al.*, 1987). No full-scale process of this new metal recovery technique has been developed to date; the mineral industry needs to be convinced of the advantages of this new technology through pilot-plant studies. Two years ago, a continuous laboratory-scale pilot plant study was carried out at McMaster University (Tsezos, 1988; Tsezos *et al.*, 1989). The results were encouraging: a 30-fold concentration of uranium was demonstrated from 300 mgU/L Denison Mines water, and a total of 12 loading-elution cycles were accomplished with no apparent loss of efficiency.

A larger scale pilot plant was built at CANMET using 100-fold more immobilized *R. arrhizus* biomass. The goals were to:

- determine the number of loading-elution cycles before the biomaterial breaks down;
- gather sufficient data for an economic assessment of the process;
- compare results at larger scale with those previously obtained at small scale;
- anticipate problems that may occur at full scale; and
- assess the effect of some parameters, such as flow rate, on column efficiency.

This work reports on the first eight loading-elution cycles of the uranium pilot plant.

Materials and Methods

Biomaterial Production and Characterization

A pure strain of *R. arrhizus* was grown and immobilized at McMaster University using a proprietary process. More details on the biomaterial production can be found in literature (Tsezos *et al.* 1989). Two types of biomaterial, produced separately and called A and B, were prepared from *R. arrhizus*. Samples of both type A and B were digested in a concentrated HNO_3-$HClO_4$ solution, and analyzed for uranium, iron , calcium, magnesium, silicon, aluminum, phosphorus, sodium, thorium and yttrium.

To determine the dry weight of biomaterial after washing, three dry samples of each type of biomaterial were weighed, placed in small glass columns, washed slowly with tap water for 24 h, then air dried for 24 h and weighed again.

Uranium isotherms were carried out on both biomaterial types using pH 4 settled mine water and pH 4 uranyl nitrate solution. Various weights of biomaterial were contacted for 10 days with 100 mL of uranium solution in 250 mL shake flasks placed in an orbital shaker at 25°C and 150 rpm. Biomaterial was recovered by filtering on a 0.45 μm Millipore filter. The filtrates were then analyzed for uranium. The initial uranium concentration of Denison Mines water was 131 mg/L and 148 mg/L after pH adjustment, and the initial uranium concentration of uranyl nitrate solution was 515 mg/L.

Chemical and Physical Analyses

Uranium analyses were carried out using a fluorimetry method. Repeated uranium analyses of the elution samples showed that method error was between 4 and 10%. The same method was used for uranium analyses of the elution samples containing uranium carbonate complexes and a large uranium precipitate. The samples were mixed before taking an aliquot and the error of the fluorimetric method was up to 13% for these samples.

Iron, calcium, magnesium, silicon and aluminum analyses were carried out by an injected coupled plasma (ICP) method. The error of this method was 5%. Sulphur, thorium, yttrium, scandium and rare earths were analyzed by high pressure liquid chromatography (HPLC). Sulphur was analyzed as sulphate. The error of the method was 2% for thorium and sulphur, and 5% for yttrium and scandium.

The biomass samples taken from the column were digested in 50 mL HNO_3–$HClO_4$ solution and the resulting solutions were also analyzed for uranium, iron, calcium, magnesium, silicon, aluminum, thorium, yttrium and scandium using the same methods described above. They were analyzed for phosphorus with an ICP method and for sodium by atomic absorption.

Radioactivity in the column was measured with a Geiger counter (Type McPhar, No. M14–79178–TC–33A). Eight fixed marks were drawn 10 cm apart on the column surface. Measurements were carried out by pressing the probe on each spot of the column surface. Background of the empty column was 8 to 10 microrems per hour (μR/h).

Continuous Pilot–Plant Experiment

The transparent acrylic column of the pilot plant was 6.4 cm in diameter and about 97 cm long. The column was filled with 880.0 g of type A biomaterial overlain with 84.5 g of type B biomaterial, because of the smaller particle size of type B. The screens were held in place by screwed caps at both ends of the cylinder. A schematic diagram of the pilot plant is shown in Figure 1. Biomaterial in the column was not used as a packed bed but as an extendible bed, meaning that the bed volume varied depending on the flow rate and the specific gravity of the biomaterial. Before the first loading–elution cycle, the 85 cm biomaterial bed was washed with Ottawa tap water for 60 h at 6.5 mL/min. After draining the column, the first loading cycle was started.

Loading Cycle

After the mine water was processed, the column was loaded in an up-flow direction with a peristaltic pump (Masterflex, 6:600 rpm). The loading flow rate was 31.5 mL/min and was monitored by a standardized flow meter placed in line after the column. The column effluent solution passed through a three-way control valve controlled by a programmable timer (Type Gralab 625). The timer energized the three-way control valve and the effluent was diverted to a programmable fraction collector (Type LKB, Superrac 2211). After a 25 mL sample was taken, the three-way control valve was energized again and the effluent was diverted to a collecting vessel. Thirty minutes after starting the sampling system, the fraction collector moved to the next position, the three-way control valve was simultaneously energized and another 25 mL sample was taken.

Up to 180 samples could be taken, meaning that the pilot plant was able to run for 90 h. If evaporation occurred, distilled water was added to the sample before it was analyzed. Every other sample was analyzed for uranium. Every eighth sample was analyzed for iron, calcium, magnesium, silicon, aluminum, thorium and yttrium. The volumes of mine water to load the column varied from 160 to 350 L, depending on the cycle.

When loading was completed, the column was drained and the biomaterial was washed with 22 L of tap water in an up-flow direction. Liquid samples were taken during washing. After washing, the column was drained and drain water was mixed with the wash water for analyses. After each loading cycle, a sample of about 25 mg of biomaterial was taken out from the top of the column. For a few cycles, some biomaterial particles passed through or around the bottom screen, offering some data from the bottom of the column.

Elution Cycle

After completing the loading cycle, the column was eluted with 25 L of 0.5 N $NaHCO_3$ solution prepared with tap water with an up-flow direction at 31.5 mL/min for the first 4 elutions and 18 mL/min for the other elutions. $NaHCO_3$ was shown to be an efficient eluant for uranium (Tsezos, 1984). During cycles 1 to 4, a 25 mL sample was taken every 5 min for the first 40 samples and then every 15 min, using the same sampling system described for the loading process. During the other cycles, samples were taken every 8 min for the first 30 samples and then every 30 min. All the samples were analyzed for uranium and some of them for the other elements.

The column was drained, washed with 20 L of tap water, and drained again. Biomaterial samples were taken from the column as described previously.

For each cycle, the total effluent solutions were analyzed for uranium, iron, calcium, magnesium, silicon, aluminum, thorium, yttrium, sulphur and scandium and were weighed. The pH was measured as well. Thus, mass balances and uptakes of 10 elements could be calculated.

Results

Chemical and Physical Characterization of Biomaterial

The chemical analyses of both biomaterial types are shown in Table 1. Type B contained more phosphorus than type A, and less calcium and iron. The weight loss of biomass after water washing was determined for both types of biomaterial. The weight loss, determined as a percentage of the initial weight, was 51.2% ± 0.6% for type A and 44.9% ± 0.7% for type B. Before starting the first loading, biomaterial in the large column was washed at 6.5 mL/min with tap water for 60 h. Analyses of wash water showed that calcium, magnesium and phosphorus were released by biomaterial during washing. It was assumed that the dry weight of biomaterial remaining in the column after washing was 50.6% less than the initial dry weight, according to the results obtained on a smaller scale.

Biosorption Isotherms

Figure 2 shows the biosorption isotherms with synthetic uranyl nitrate solution for both type A and type B biomaterial, and Figure 3 shows the biosorption isotherms with Denison Mines water. The uranium uptake capacities of type B were slightly higher than those of type A with a synthetic uranium solution (see Figure 2). Uranium uptakes as high as 300 mgU/g of dry biomass were obtained with type B. Biomaterial was not washed before use, meaning that the real uranium uptakes were twice as much as uranium uptakes shown in figures 2 and 3. With Denison Mines water, the uranium uptake capacities of biomaterial were not strongly dependent on equilibrium uranium concentration, at equilibrium concentrations less than 100 mgU/L. There was no significant difference in uranium uptake between both types of biomaterial using mine water.

Denison Mines Water Analyses

The mine water used in this work was a biolixiviant from Denison Mines bacterial leaching stopes. The chemical composition of this mine water varied with time since bacterial leaching is adversely affected by the lower winter temperatures. A common mine water pH was 2.5 and uranium concentrations varied between 95 and 150 mg/L. An example of chemical composition of mine water is in Table 2, with the range of concentration variations.

To precipitate part of thorium and most iron, and to reach the optimum uranium biosorption pH of *R. arrhizus*, mine water was adjusted to pH 4.0 or 4.1 with concentrated NaOH in a 200 L tank. After pH adjustment, if clear, the solution was separated from the precipitate. If not, the pH was adjusted again to 4.0. The pH of clear solution was generally lower than 4.0 and thus, the pH of the pilot plant feed was between 3.7 and 4.0. The clear solution was analyzed several times for uranium, iron, calcium, magnesium, silicon, aluminum, thorium, yttrium, scandium and sulphur during loading of the column. A typical mine water composition at pH 4 is given in Table 2. Generally, at least 85% of the thorium precipitated at pH 4.0. Uranium concentration of clear solution was often about 10% less than the as-received mine water. Except for one batch of mine water, between 98 and 99.9% of iron precipitated during pH adjustment. For loading-elution cycles 1 to 8, the clear solution was not filtered before being used.

Interpreting results was difficult because of the various compositions of the feed.

Pilot-Plant Results: Cycles 1 to 5

Loading-elution cycles 1 to 5 were carried out without interruption as described previously. Figure 4 is a typical loading breakthrough curve for uranium. Until the breakthrough point was reached, 100% uranium was adsorbed on biomaterial. Then, the effluent uranium concentration increased gradually until effluent uranium concentration was equal to influent uranium concentration. From this point, and for all the loading breakthrough curves, a big variation of effluent uranium concentration was observed and probably some of the uranium previously adsorbed was released by biomaterial. Figure 5 shows the first five loading breakthrough curves for uranium. The uranium uptake capacity of biomaterial decreased from cycle 1 to cycle 4. From Table 3, the uranium uptake, calculated by mass balance with the initial weight of biomaterial, was 60% less for cycle 4 compared with cycle 1. However, uranium uptakes measured on biomaterial samples taken out at the top of the column were constant after cycle 1 (see Table 3). This means that after cycle 1, the decrease of uranium uptake capacity was because of modification of the uranium uptake capacity of biomaterial at the bottom of the olumn. A biomaterial sample was taken from the bottom after loading 5 and contained only 16.2 mgU/g, compared with 37.8 mgU/g at the top. A pronounced reddish colour at the bottom of the column (20 cm) was also observed.

For cycle 1, the difference between the uranium uptake calculated by mass balance (27.0 mgU/g) and the uranium uptake measured in biomaterial from the top of the column (60.0 mgU/g) showed that at least 50% of the initial dry weight of biomaterial was lost during water washing and first loading.

After loading the column with uranium, it was washed with 22 L of tap water. About 5% of the uranium was lost in this wash water.

Elution cycles were carried out with 25 L of 0.5 N $NaHCO_3$ solution. A typical elution curve is presented in Figure 6. High peak concentrations were obtained, up to 13.5 gU/L. For cycles 1 to 5, the uranium peak concentration was higher than 9.4 gU/L. Elution recoveries were between 73 and 105% and generally greater than 90% (see Table 3).

Results of biomaterial analyses at the end of the loading-elution cycles confirmed that uranium remaining in the column was less than 0.25 mgU/g.

During loading, iron, calcium, magnesium, silicon, aluminum, thorium, europium and yttrium concentrations were measured in the effluent solutions. Typical loading curves for these elements are in figures 7 to 9. The following chart summarizes the behaviour of the analyzed elements in Denison Mines water during loading.

Elements Released by Biomass	Elements Adsorbed	Elements Adsorbed and then Partially Desorbed	Elements not Adsorbed
magnesium, calcium	iron, thorium	aluminum, yttrium, rare earths	silicon, sulphur (may be partially adsorbed)

Thorium was completely adsorbed by biomaterial (see Figure 7) and aluminum was completely adsorbed within the first 10 L of feed (see Figure 8).

Table 1 shows that during the first five cycles, the concentrations of iron, aluminum, thorium and yttrium increased in the biomaterial, especially at the bottom of the column. At the end of loading 5, there was 0.63% iron, 0.05% thorium and 1.2% aluminum at the top of the column and 5.20% iron, 3.62% thorium and 0.2% aluminum at the bottom. The behaviour of aluminum was different from that of iron and thorium. Typical elution curves for iron, calcium, aluminum, yttrium, sulphur and thorium are in figures 10 and 11. The following chart summarizes the behaviour of the elements studied during elution.

Elements Released by Biomass	Elements Stripped	Elements not Stripped (Previously Adsorbed)	Elements not Stripped
magnesium	thorium	iron, aluminum, yttrium	calcium, silicon, sulphur

Table 1 shows that a significant amount of iron and aluminum remained in the column at the end of elution 5.

Radioactivity measurements in the column showed that:

- radioactivity of biomaterial increased during loading;
- radioactivity was three times less at the top of the column than at the bottom; and
- radioactivity decreased during elution but did not decline to the background level.

Figure 12 represents in three dimensions the correlation between radioactivity in the column, different column heights, from 10 to 80 cm, and the effluent volume during elution 2. The elution breakthrough curve of cycle 2 for uranium is also presented in this graph. For this last curve, the y-axis, corresponding to the effluent uranium concentration, is not reported in the figure. Radioactivity was lower at the end of elution 2 and a wave of radioactivity was observed, corresponding to the uranium peak concentration. It means that some radioactive compounds were eluted at the same time as the uranium. Thus, radioactive measurements are a way to understand the movement of uranium through the column during elution or loading.

EDTA Washing

To resolve the problem of reduction in uptake capacity and to reduce the amount of iron and aluminum adsorbed in biomaterial, the column was washed after elution 5 with a 0.1 N EDTA solution at 40°C and 13.8 mL/min. EDTA was chosen because it could be used in a 3 to 8 pH range without damaging the biomaterial. The EDTA elution curves (see Figure 13) show that part of the aluminum, iron and calcium was stripped from the column. At the top of the column, biomaterial contained 0.45% iron, 0.27% thorium and 0.81% aluminum after washing with EDTA. Radioactivity measurements after EDTA washing showed that the radioactivity level in the whole column was back to the initial level.

Pilot-Plant Results: Cycles 6 to 8

The loading breakthrough curve for uranium of cycle 6 was the same as that of cycle 3, meaning that EDTA washing allowed an increase in the uranium uptake capacity of biomaterial (see Figure 14). Uranium uptake capacity of biomaterial decreased from cycle 6 to cycle 8.

The uranium peak concentrations during elution were close to 8 gU/L and the elution recoveries were in the same range as those of cycles 1 to 5.

The behaviour of the other elements was similar to their behaviour during the previous cycles.

Finally, in the first eight cycles, a concentrated uranium solution was obtained. As an example, the uranium analyses of raw biolixiviant, processed pH 4 mine water and concentrated uranium solution of cycle 2 are given in Table 2. Iron, calcium, magnesium, silicon and aluminum were present in this concentrate at very low concentrations compared with the concentrations of raw biolixiviant. The concentrate contained thorium but less than 15% of the thorium present in the raw biolixiviant. If only 8 L of $NaHCO_3$ solution had been used to strip uranium from the column, a 40-fold uranium concentration would have been obtained. For cycle 8, the concentration of the first 2.8 L of solution was 3090 mgU/L (i.e., a concentration factor of 26.5). This indicates that the process is selective for uranium.

Discussion

The results of the pilot plant have to be compared with those obtained previously on a smaller scale (Tsezos, 1988). From this study, it was concluded that the uranium uptake capacity of biomaterial was stabilized after the second cycle. These results were confirmed on a larger scale at the top of the column, but not at the bottom. Because an up-flow direction was used, biomaterial at the bottom of the column was affected more by the competing ions in mine water than biomaterial at the top. For the eight loading cycles the feed solution was not filtered and some accumulation of iron precipitate around biomaterial particles might have occurred in addition to iron biosorption. Aluminum seemed also to be an efficient competing ion for uranium. The uranium uptake capacity of biomaterial used in the pilot plant was lower (30-60 mgU/g) than that used on a smaller scale (43-72 mgU/g).

Iron and aluminum were not eluted at pH 8 with $NaHCO_3$ but were partially eluted with EDTA. EDTA washing was not optimized and an optimization of the flow rate and of the concentration may improve the uranium uptake capacity of biomaterial. The flow rate was chosen to correspond to a residence time of solution in the biomaterial bed of about 30 min; although the flow rate was adequate, a higher flow rate could probably be used with the same efficiency.

The uranium peak concentration obtained at smaller scale was 5 gU/L for the first cycle and less than 4 gU/L for the other cycles (Tsezos, 1988). On the larger scale, the uranium peak concentrations were much higher, because of the larger depth of the biomaterial bed. This suggests that, on a much larger scale, very high uranium concentrations may be obtained. Even after eight cycles, a 26.5-fold uranium concentration was possible using unfiltered processed mine water. For the first three cycles, a 40-fold uranium concentration could have been obtained by reducing the elution volume.

Conclusions

From the results of the pilot plant so far, the following conclusions can be drawn:

- at the end of the loading cycles, some uranium may be desorbed;
- for the eight loading–elution cycles, a 26– to 40–fold uranium concentration was obtained;
- uranium concentration is selective and the eluant effluent contains very low concentrations of iron, calcium, magnesium, silicon, aluminum, yttrium and sulphur;
- all the uranium adsorbed can be eluted with $NaHCO_3$;
- after a few cycles, uranium uptake capacity of biomaterial is reduced at the influent of the column;
- iron, aluminum and some radioactive compounds may reduce uranium uptake capacity; and
- pH 4 mine water has to be filtered before use.

Despite the decrease of uranium uptake capacity, the use of immobilized biomass is an efficient means to remove and concentrate uranium from Denison Mines water. Results at the pilot scale are encouraging, but more work is necessary to understand the loss of efficiency of biomaterial and to continue optimization of the process parameters.

Selectivity of the process is the result of pH adjustment of the mine water, the selective uranium uptake capacity of *R. arrhizus* biomass and the selectivity of elution using $NaHCO_3$.

Such a selective process could lead to a larger scale application in the next few years.

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TABLE 1 : Biomaterial analyses for the first 5 cycles

Type A = Biomaterial called type A
Type B = Biomaterial called type B
L = Biomaterial sample taken at end of loading run
E = Biomaterial sample taken at end of elution run
(BOT) = Additional sample taken at bottom of column

	U(mg/g)	Fe(%)	Ca(%)	Mg(%)	Si(%)	Al(%)
TYPE A	0.001	0.0352	0.679	0.289	0.034	0.0075
TYPE B	0.001	0.0270	0.378	0.2290	0.042	0.011

	P(%)	Na(%)	S(%)	Th(%)	Y(%)
TYPE A	1.16	0.055	<0.008	<0.005	<0.001
TYPE B	1.43	0.059	<0.008	<0.005	<0.001

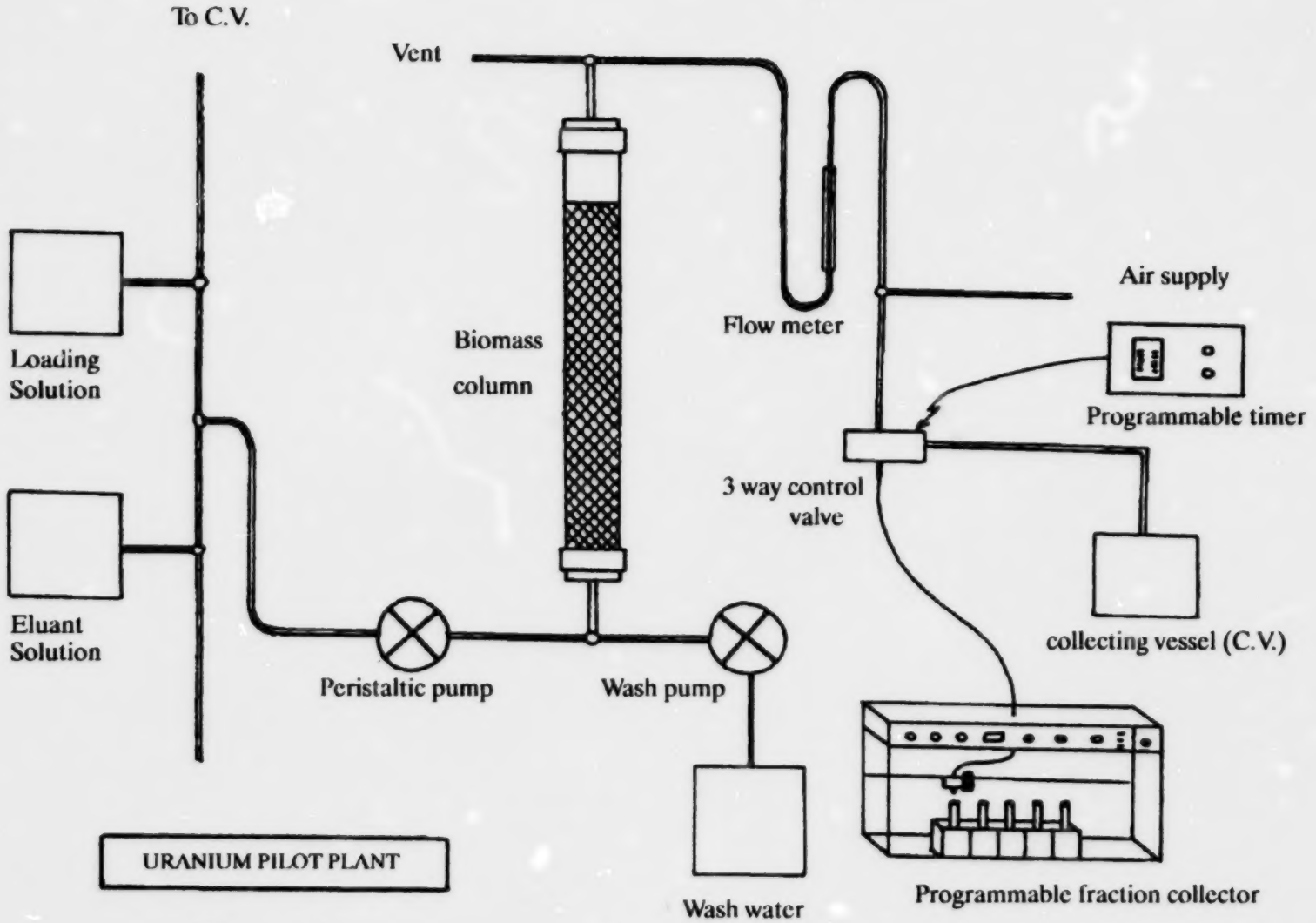
	U(mg/g)	Fe(%)	Al(%)	Th(%)	Y(%)
L 1	60.4	0.0360	0.124	<0.005	<0.001
E 1	0.15	0.185	0.106	<0.005	<0.001
L 2	34.0	0.17	0.62	0.029	<0.001
E 2	0.6	0.33	0.68	0.095	0.01
L 3	38.8	0.19	1.10	0.059	0.0068
E 3	0.3	0.24	<4.7	0.012	0.0068
L 4	36.4	0.37	1.4	0.52	0.03
E 4	0.2	0.27	1.1	0.29	<0.005
L5	37.8	0.63	1.2	0.05	0.037
L5(BOT)	16.2	5.2	0.2	3.62	0.04
E5	0.2	1.1	1.4	0.028	0.012
EDTA E5	0.2	0.45	0.81	0.027	<0.005
EDTA E5(BOT)	0.2	2.4	0.96	0.013	<0.005

TABLE 2

	Concentration range (mine water at pH 2.5)	Concentration range (mine water at pH 3.9)	Mine water before pH adjustment (pH=2.5) Cycle 2	Mine water after pH adjustment (pH=3.9) Cycle 2	Elution effluent Cycle 2
U (mg/l)	95–160	99–160	99	90	643
Fe (mg/l)	259–501	0.6–7	352	0.7	0.5
Ca (mg/l)	200–280	200–280	235	242	2.8
Mg (mg/l)	13–20	13–20	15.6	15.7	1.3
Si (mg/l)	20–26	20–26	22	20.7	2.2
Al (mg/l)	30–53	27–45	30	27.3	0.4
Y (mg/l)	14–26	14–21	14.6	14.3	<0.5
S (mg/l)	520–770	520–730	770	714	200
Sc (mg/l)	<0.5	<0.5	<0.5	<0.5	<0.5
Th (mg/l)	44–181	0.6–51	44.1	2.92	23.6

TABLE 3

	Uranium Uptake Calculated by mass balance (mg/g)	Uranium Uptake Measured from biomass (top of the column) (mg/g)	Elution Uranium Recovery (%)
Cycle 1	27.0	60.4	100.4
Cycle 2	17.7	34.0	90.4
Cycle 3	16.6	38.8	76.5
Cycle 4	10.7	36.4	104.7
Cycle 5	12.6	37.8	98.4
Cycle 6	16.2	29.1	73.6
Cycle 7	10.0	32.9	96.2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Eluant
Solution
collecting vessel (C.V.)
Peristaltic pump
Wash pump
URANIUM PILOT PLANT
Wash water
Programmable fraction collector

Figure 1.

BIOSORPTION ISOTHERMS
Synthetic solution: 515.3 mgU/I

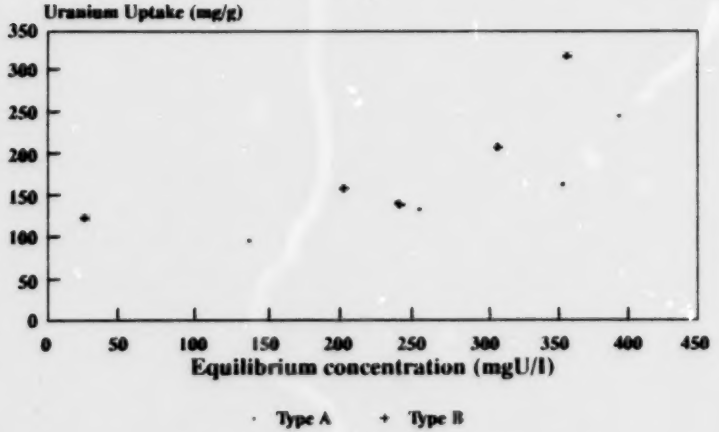

Figure 2.

BIOSORPTION ISOTHERMS
Denison Mine Water: 148 & 131 mgU/I

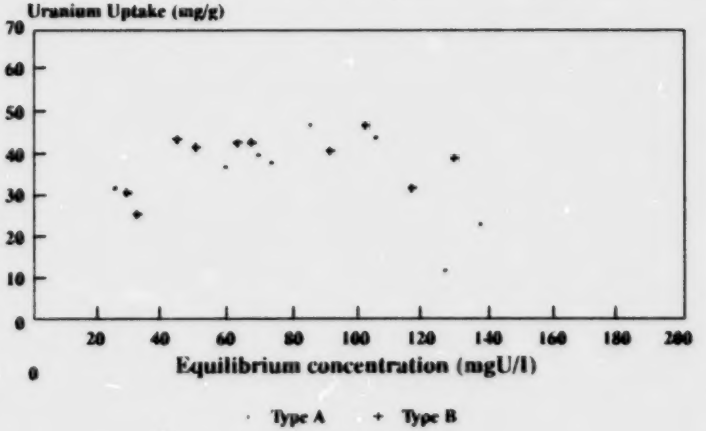

Figure 3.

LOADING BREAKTHROUGH CURVE

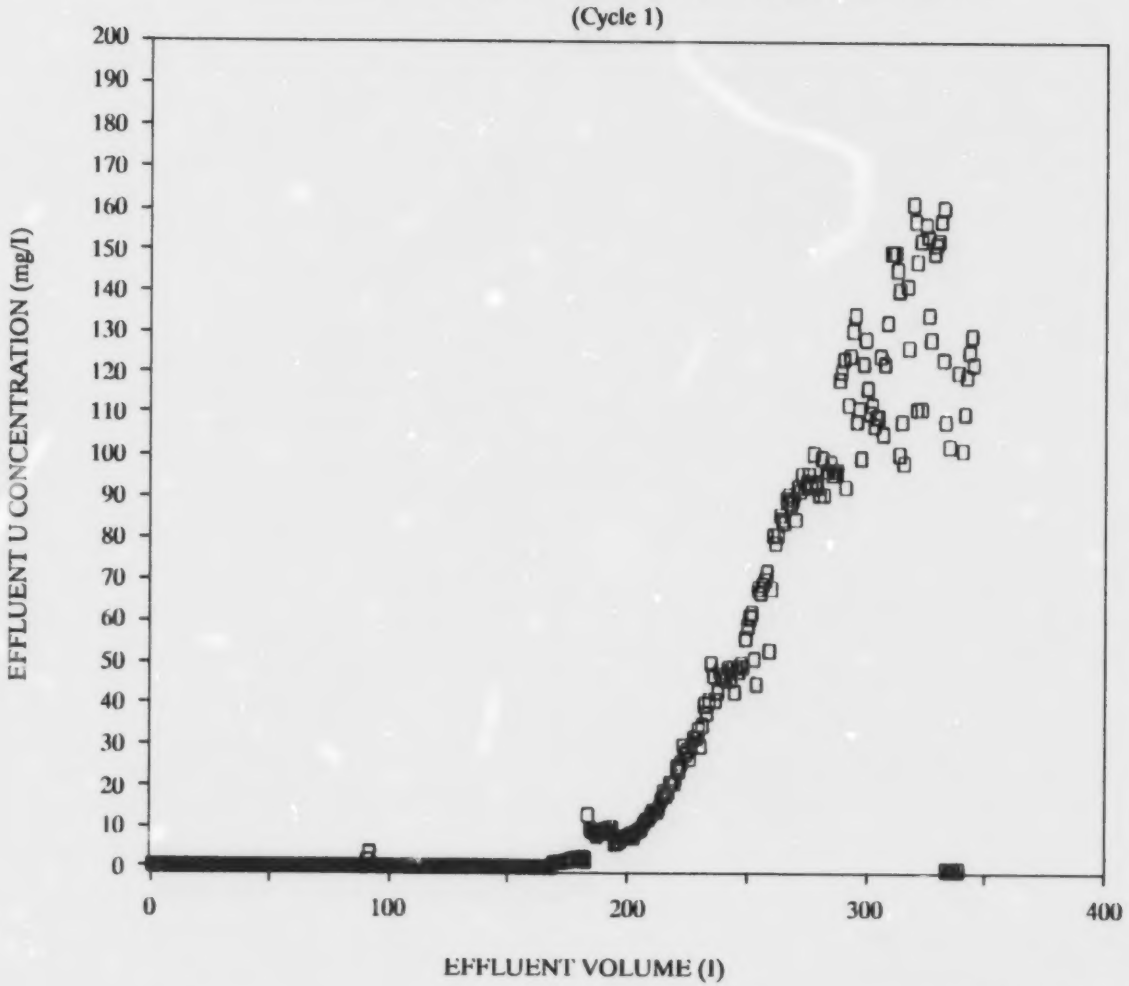

Figure 4.

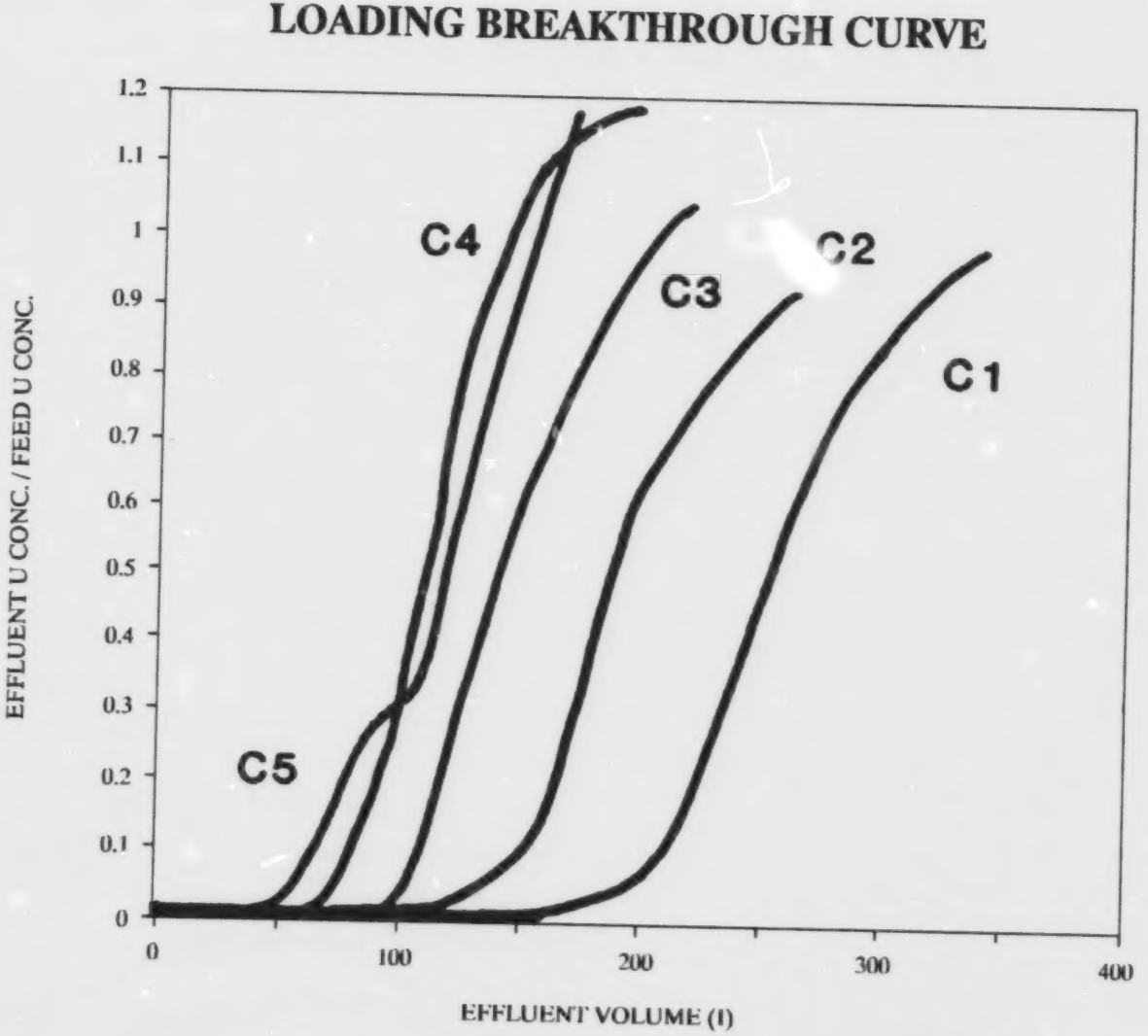
LOADING BREAKTHROUGH CURVE
EFFLUENT U CONC. / FEED U CONC.
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
C4
C3
C2
C1
C5
0
100
200
300
400
EFFLUENT VOLUME (l)

Figure 5.

ELUTION BREAKTHROUGH CURVE
(Cycle 2)

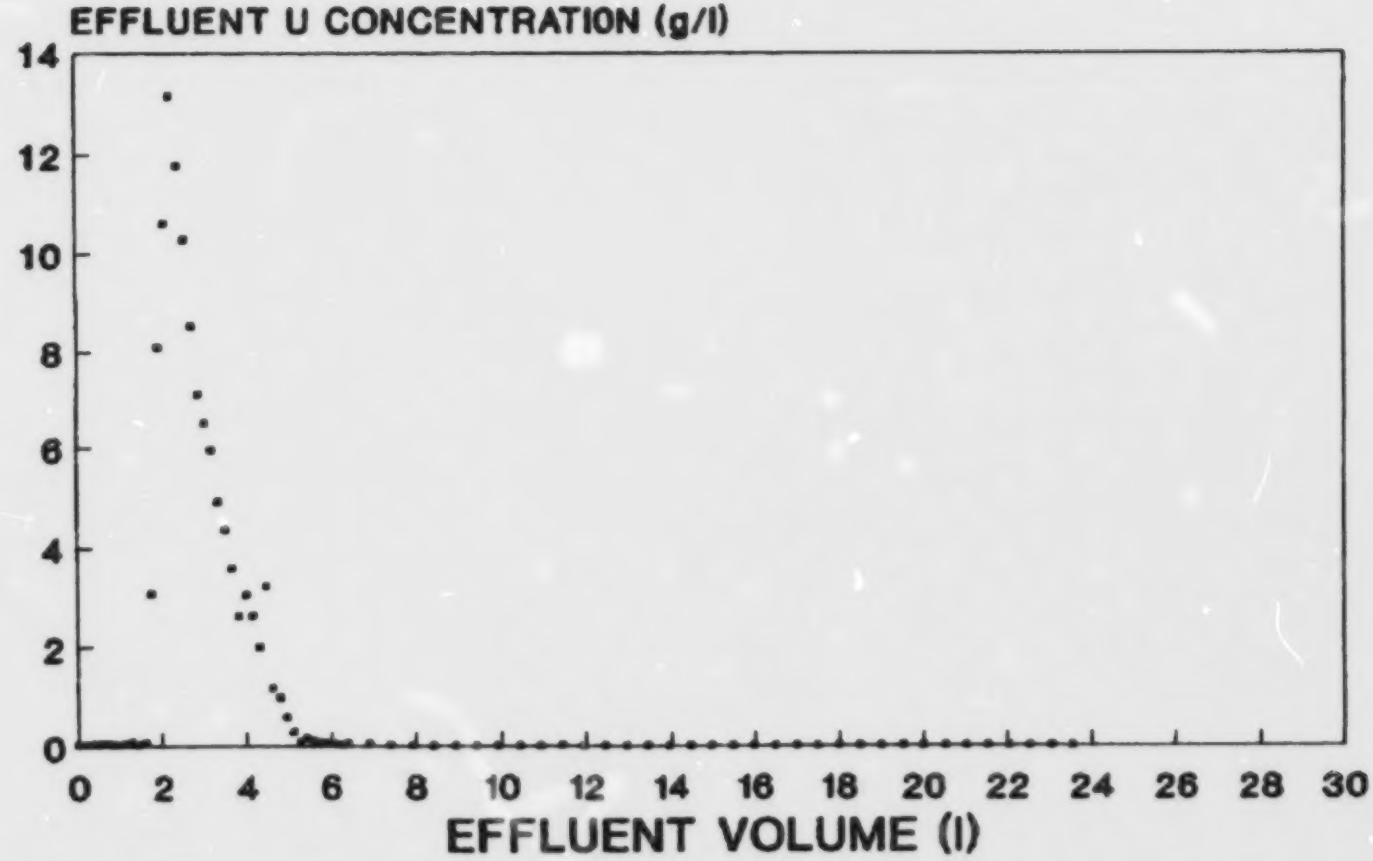

Figure 6.

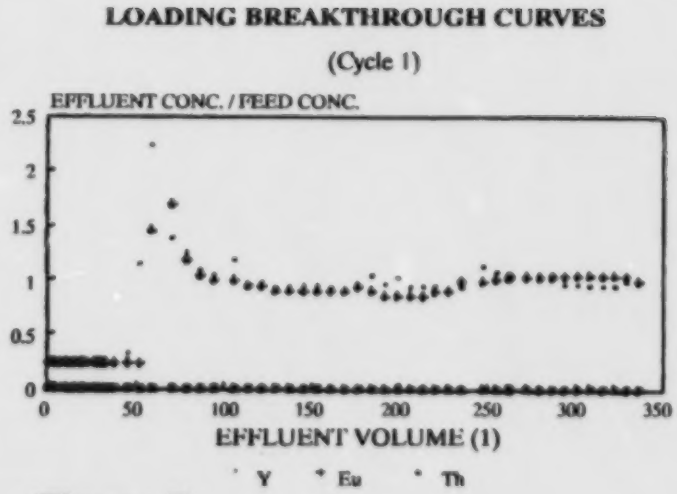

Figure 7.

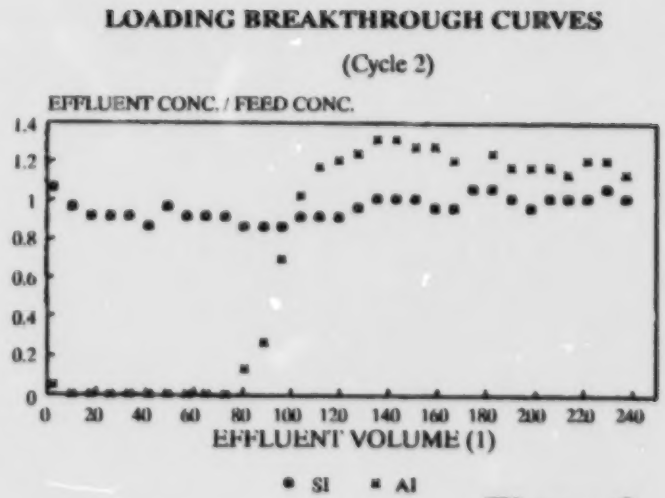

Figure 8.

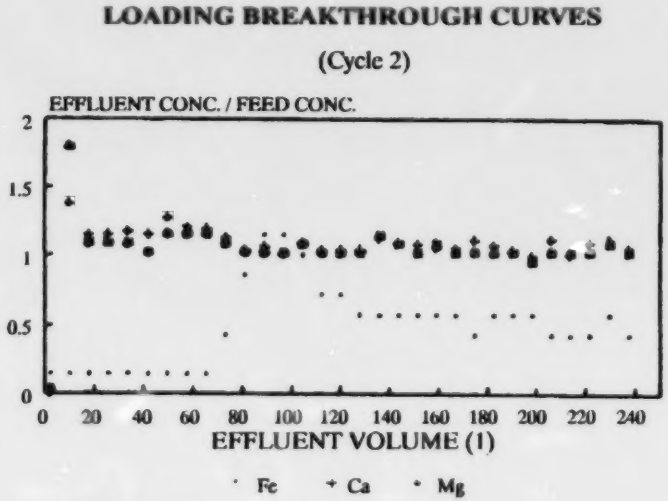

Figure 9.

ELUTION 3
Fe – Ca – Al

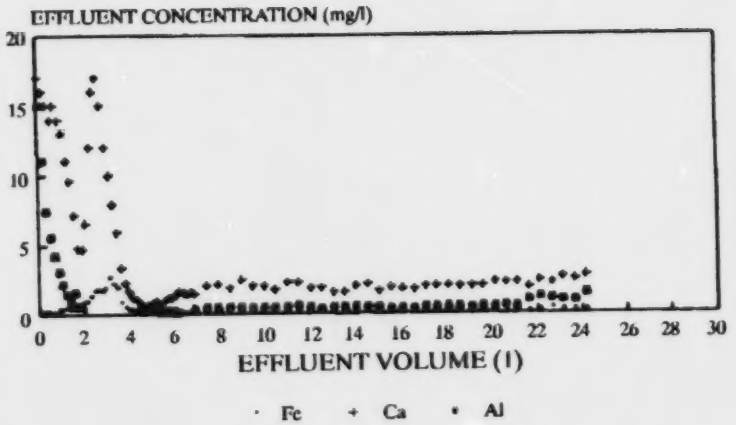

Figure 10.

ELUTION 1
Al – Th – Y – S

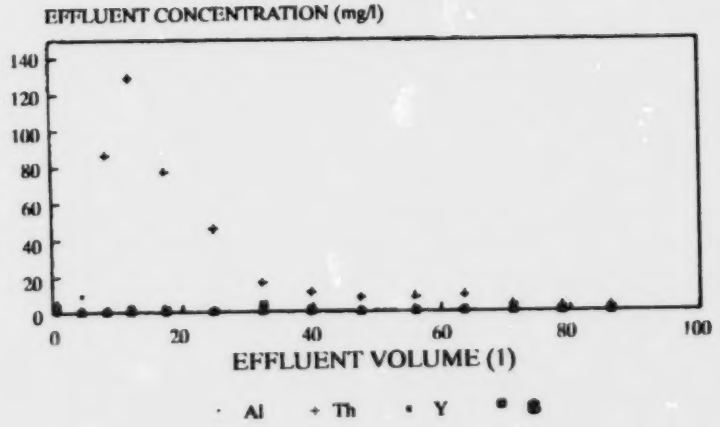

Figure 11.

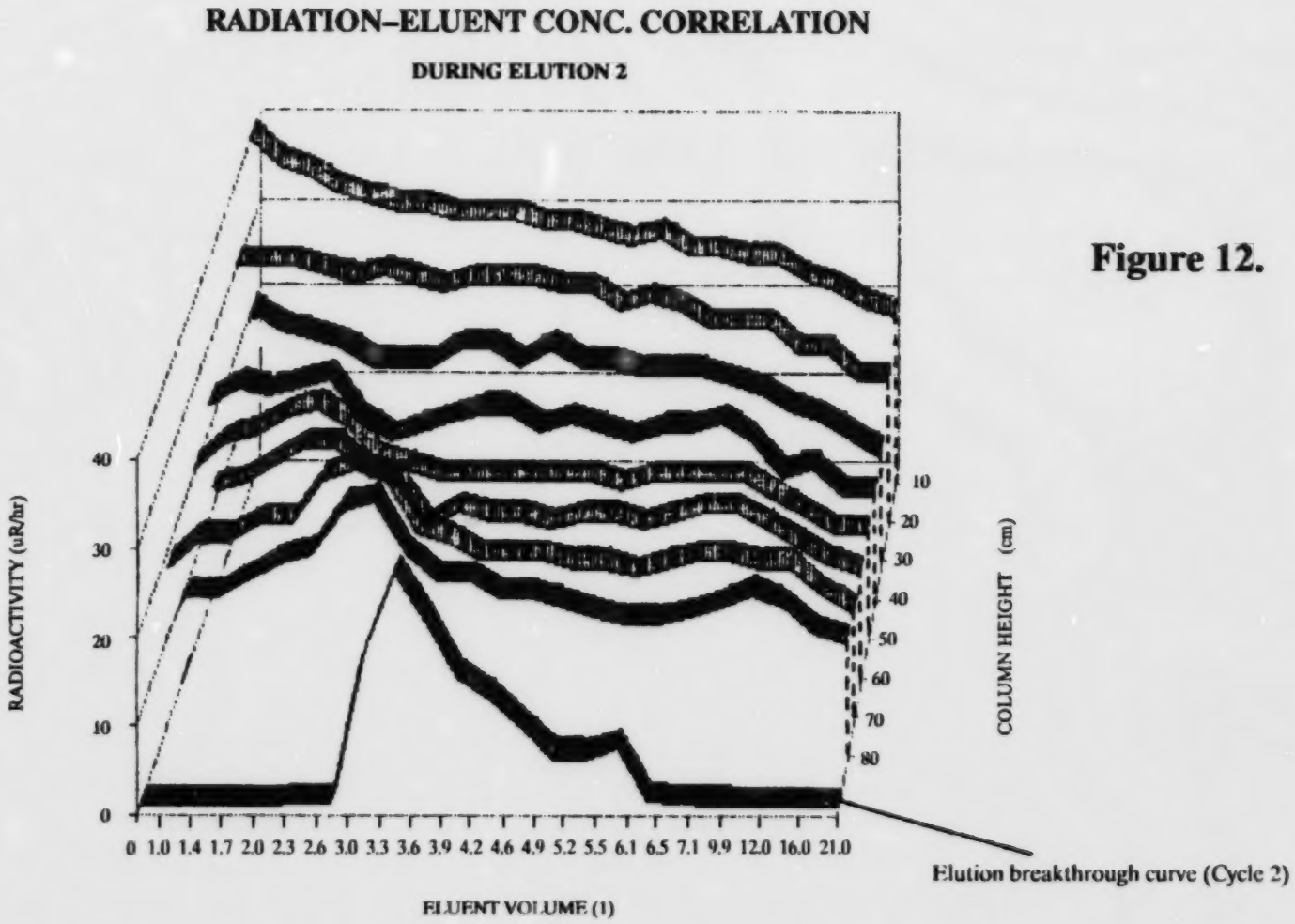
RADIATION–ELUENT CONC. CORRELATION
DURING ELUTION 2
RADIOACTIVITY (uR/hr)
0
10
20
30
40
0 1.0 1.4 1.7 2.0 2.3 2.6 3.0 3.3 3.6 3.9 4.2 4.6 4.9 5.2 5.5 6.1 6.5 7.1 9.9 12.0 16.0 21.0
ELUENT VOLUME (l)
COLUMN HEIGHT (cm)
10
20
30
40
50
60
70
80
Elution breakthrough curve (Cycle 2)

Figure 12.

EDTA ELUTION CURVES

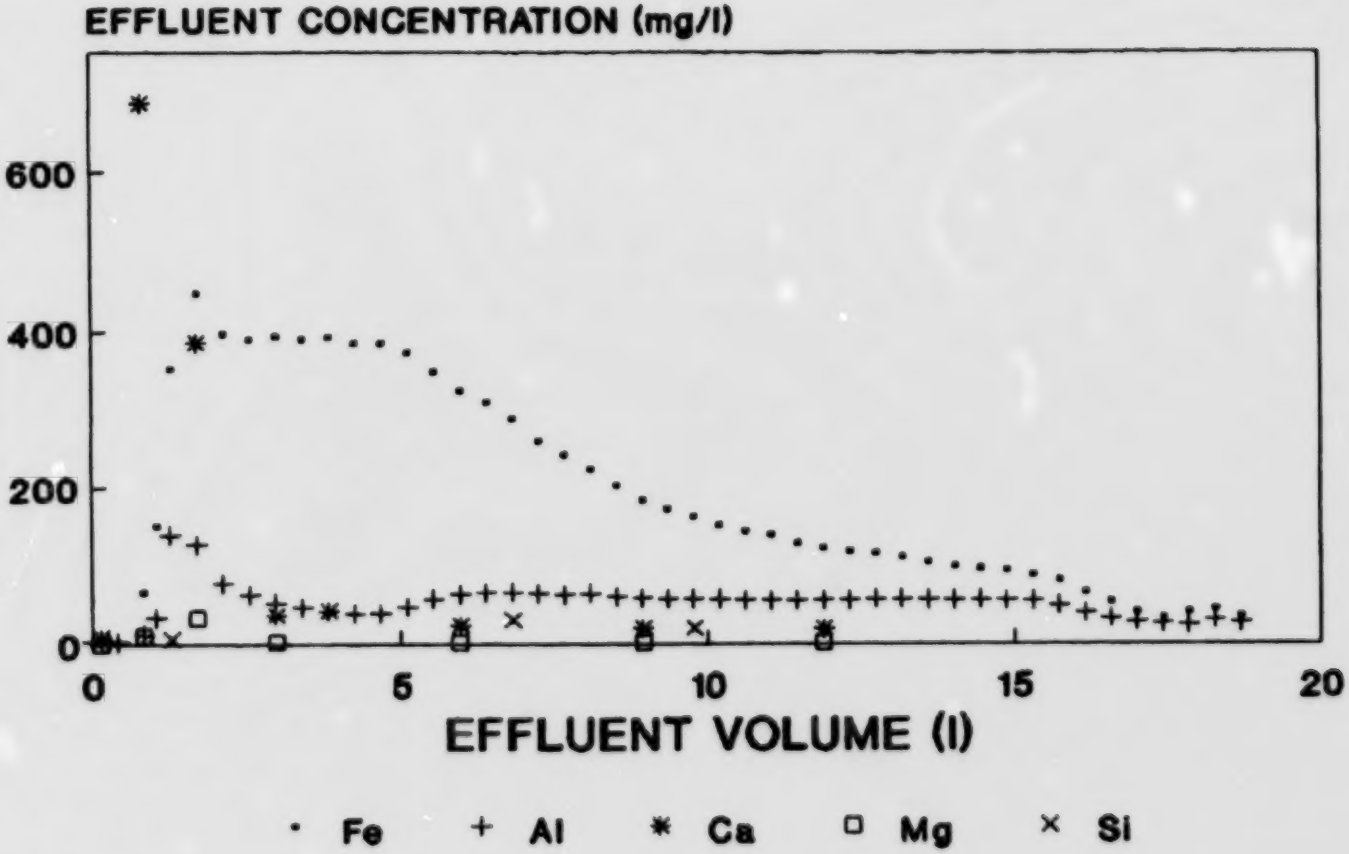

Figure 13.

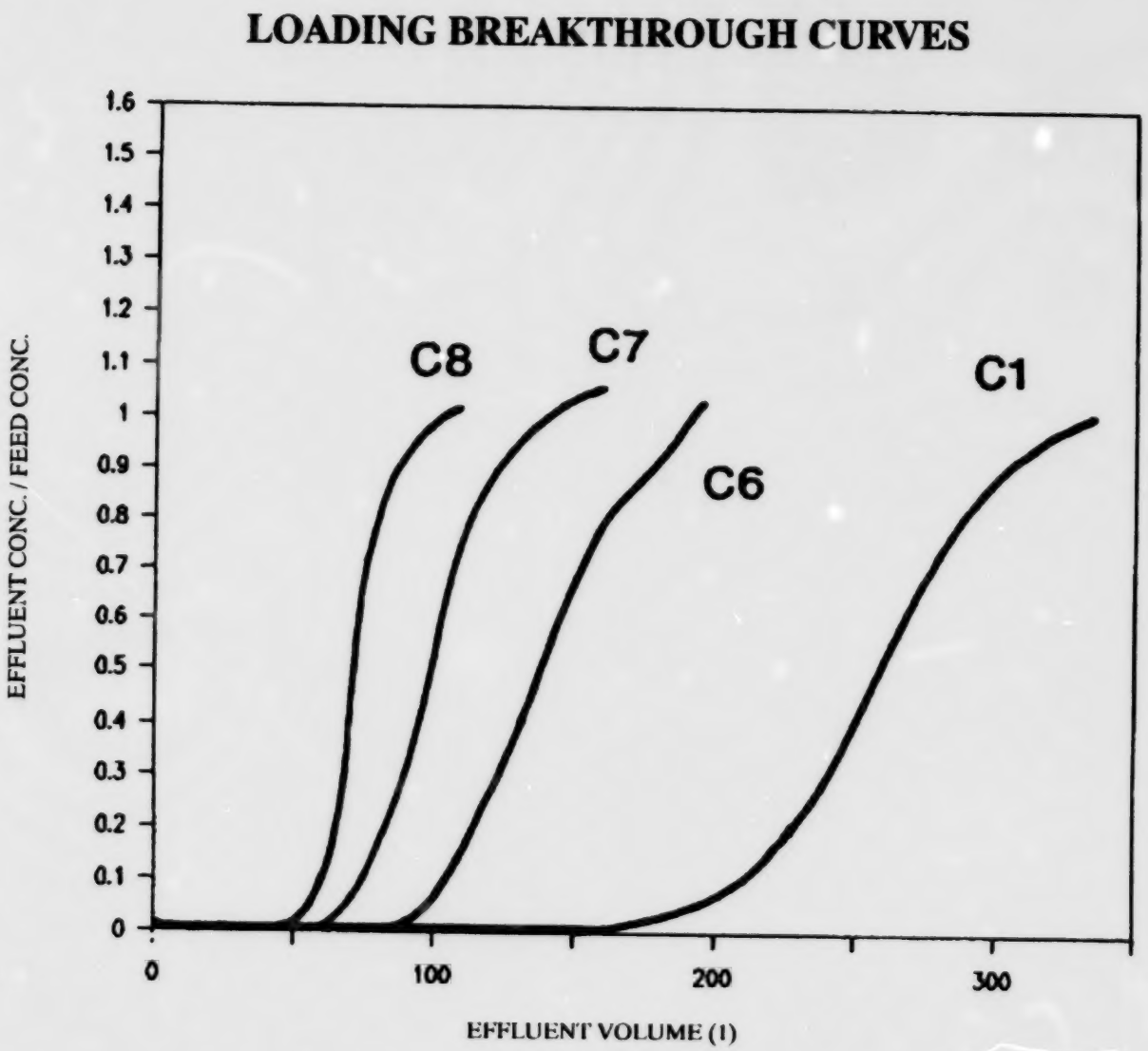
LOADING BREAKTHROUGH CURVES
EFFLUENT CONC. / FEED CONC.
1.6
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
C8
C7
C6
C1
0
100
200
300
EFFLUENT VOLUME (l)

Figure 14.

Acid Mine Drainage Research in Canada

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Ottawa, Ontario

and

K. Ferguson
Environment Canada (Pacific and Northwest Region)
Vancouver, British Columbia

Abstract

Acid mine drainage (AMD) is the largest single environmental problem facing the mining industry today. Technologies to prevent AMD from occurring in waste rock piles and tailings sites, and on the walls of open pits and underground mines, need to be developed and demonstrated. Two organizations in Canada have accepted this challenge: the national Mine Environment Neutral Drainage (MEND) program and the British Columbia Acid Mine Drainage (BC AMD) Task Force. This paper summarizes the activities of these two organizations.

Keywords: Tailings, waste rock, sulphide oxidation, MEND, BC AMD Task Force, reactive wastes, acid mine drainage.

Résumé

Les eaux de drainage acides constituent le plus grand problème auquel a à faire face l'industrie minière d'aujourd'hui. Il faut concevoir et démontrer des technologies qui permettent d'empêcher l'acidification des eaux dans les stériles et les décharges de résidus et sur les parois des mines à ciel ouvert et souterraines. Deux organismes au Canada ont accepté de relever ce défi: le Programme fédéral de neutralisation des eaux de drainage dans l'environnement minier et l'Acid Mine Drainage Task Force. Le présent document présente un résumé des activités de ces deux organismes.

Mots-clés : résidus, stériles, oxydation des sulfures, NEDEM, BC AMD Task Force, déchets réactifs, eaux de drainage acides.

Introduction

For more than 20 years, the mining industry and government conducted research on methods of establishing sustainable vegetative growth on tailings and waste rock. It was believed that this technology would alleviate acid drainage problems from these sites, thus allowing mining companies to meet environmental requirements after closing. Very successful vegetation methods were developed, and many sites supported vegetation. After several years of observation, however, the drainage quality from vegetated sites has not improved, and mining companies are faced with the prospect of continuing to operate and maintain lime treatment plants for potentially hundreds of years. This is clearly an unacceptable solution to the problem, and new reclamation technology must be developed and demonstrated.

In response to the need to conduct research on acid mine drainage (AMD), an industry-initiated task force was assembled in 1986. The task force consisted of a steering committee and technical working group, with representation from the mining industry, CANMET, Environment Canada, British Columbia, Manitoba, Ontario, Quebec and New Brunswick. The task force recommendations were published in July 1988 (CANMET, 1988), and are being implemented by a tripartite consortium known as the Mine Environment Neutral Drainage (MEND) program. A second group in Canada, the British Columbia Acid Mine Drainage (BC AMD) Task Force, is also coordinating research into the problem of AMD.

This paper summarizes the extent of AMD in Canada and the research programs under way (MEND and BC AMD) to develop and demonstrate new cost-effective technologies.

What Is Acid Mine Drainage?

Most Canadian base metal, precious metal and uranium mines contain sulphide minerals, either in the ore or the surrounding waste rock. When these sulphide minerals, particularly pyrite and pyrrhotite, are exposed to oxygen and water, they begin to oxidize almost immediately. In the absence of calcareous materials, the initial chemical reactions produce acid and liberate heavy metals associated with the waste deposit. As the reactions continue, temperature and acidity increase, resulting in an increased rate of reaction. Between pH levels of 2 and 4, bacteria and ferric iron catalyze the reactions, and rates can be 20 to 100 times faster than the original chemical reaction rate (Knapp, 1987). Rainfall and snowmelt flush the toxic solutions from the waste sites into the downstream environment.

AMD may contain very high concentrations of sulphate, ferrous iron and base metals such as lead, copper, nickel, zinc or silver. It also exhibits pH values below 7. If AMD is left uncollected and untreated, the drainage could contaminate groundwater and local watercourses, damaging plants, wildlife and fish.

At active mine sites (and some inactive mine sites), mining companies operate comprehensive systems to collect and treat effluents and seepage from all sources. These facilities, when well operated and maintained, prevent a downstream environmental effect. However, acid generation may persist for hundreds of years after a mine closes. Operating treatment plants for long periods is not desirable.

Dimensions of the Problem

Between 1984 and 1987, CANMET and industry cosponsored two projects to define the extent of acid-generating mine waste at base metal mining operations (Monenco, 1984; Nolan, 1987). British Columbia, Saskatchewan, Manitoba, Ontario, Quebec, New Brunswick, Newfoundland, Yukon and Northwest Territories all have operating or abandoned acid-generating waste sites, with a total area of more than 15,000 ha (37,000 acres). These wastes are mostly the accumulation of 40 years of mining since the Second World War. It seems reasonable to assume that the mining of lower grade ores, together with the likelihood of increasing annual mineral production, could lead to the accumulation of an equal quantity of acidic tailings and waste rock over the next 20 years.

The above surveys do not represent the entire Canadian inventory, since they did not include gold mines, coal mines, uranium mines or abandoned mine sites that are now the responsibility of the Crown. The two provinces with the longest history of mining, Ontario and Quebec, have recently completed surveys on their abandoned mine sites. In Ontario, 100 abandoned mine sites were identified and 20 pose an AMD problem. The 20 sites contain 55,000,000 tonnes (60,000,000 tons) of reactive sulphide tailings over a surface area of 830 ha (2,030 acres). Quebec has about 107 abandoned mine sites. Of these sites, 21 have been classified as hazardous waste sites because of AMD. The total area of these 21 sites was estimated at 4,500 ha (11,110 acres).

The cost of stabilizing reactive wastes is highly site-specific, varying greatly from site to site. Under the most difficult conditions and with existing, but unproven technology, the costs of stabilizing some sites have been estimated to be as much as $410,000 per hectare. Applying an average cost of $125,000 per hectare to the existing and future accumulation of acid-generating waste, the costs of reclamation at non-ferrous metal mine sites is $3 billion over the next 20 years. Funds required to deal with abandoned sites where liability cannot be established, and where the mineral rights have reverted to the crown, are estimated to be about $1 billion.

The MEND Program

MEND is a cooperative research organization sponsored, financed and administered by the Canadian mining industry, the federal government, and the provinces of British Columbia, Manitoba, Ontario, Quebec and New Brunswick. The MEND organization includes a board of directors, a management committee and six technical committees.

The overall objectives of MEND are to:

- provide a comprehensive scientific, technical and economical basis for the mining industry and government agencies to predict, with confidence, the long-term management requirements for reactive tailings and waste rock; and
- establish techniques for operating and abandoning acid-generating tailings and waste rock disposal areas in a predictable, affordable, timely and environmentally acceptable manner.

Using these overall objectives, the technical committees will conduct research, as described by the following guidelines.

Prediction

The Prediction Committee will work on developing improved analytical protocols for identifying acid-generating waste materials and predicting the concentration of pollutants to be expected in drainage waters from these wastes. The committee will initiate research into the fundamental chemistry and reaction mechanisms of sulphide waste materials. A detailed understanding of the hydrogeochemistry of waste sites and the mineralogy of reaction products are essential parts of this work.

This research should result in an improved understanding of the acid-generating process, methods to predict whether a mine site will produce AMD, and computer models to simulate components of the acid generation and contaminant-migration process in tailings and waste rock.

Prevention and Control

The Prevention and Control Committee will demonstrate the suitability of various closure alternatives for preventing AMD formation in tailings and waste rock. The closure systems that will be examined will include the use of wet barriers and dry barriers.

Although all committees must keep abreast of new technology, it is especially true for the Prevention and Control Committee. Reductions in closeout costs depend mostly on the efforts of this committee.

Treatment

The Treatment Committee will examine and demonstrate the feasibility of new effluent treatment systems for use during operations and when a waste site is closed. Passive treatment systems, such as wetlands, may be used downstream from decommissioned waste sites, but fundamental research is needed to prove that these systems are effective and reliable.

The committee will also examine advanced treatment methods and methods for stabilizing heavy metal hydroxide sludges.

Monitoring

The Monitoring Committee is responsible for all aspects of site monitoring, including developing field sampling manuals. The committee will examine advanced monitoring methods, including remote sensing and biosensors.

Technology Transfer

All research results must be effectively communicated to industry, government agencies and the public if the program is to succeed. The Technology Transfer Committee is responsible for this task. The committee will maintain a computerized database of AMD literature, distribute the literature as required, organize seminars, and communicate with interested parties.

International Liaison

This committee will work with international organizations, collect information on international AMD research, and review and disseminate this information to MEND participants. This is CANMET's mandate for the federal government. CANMET has already developed contacts with the United States, Australia, Finland, Sweden and Norway. The Mining Association of Canada has developed contacts with the American Mining Congress, the Australian Mining Congress and others. These and other contacts will be nurtured through the committee.

To meet these objectives, the Management Committee estimates that $12.5 million in research is needed. Table 1 shows the intended distribution of the research funds.

Table 1
Summary of MEND Projects

1. Prediction	$ 3 765 000
2. Prevention and Control	$ 5 705 000
3. Treatment	$ 1 285 000
4. Monitoring	$ 385 000
5. Technology Transfer	$ 225 000
Contingency	$ 1 135 000
Total Program	**$12 500 000**

Research will be conducted through contracts issued to universities and consultants, and through the contribution of work-in-kind credits from government research laboratories and mining companies. MEND program results will be available through the CANMET publications section.

Summary of Projects Under Way

Table 2 is a list of the 20 MEND projects approved and under way, or completed, as of September 1989. The total budget for the projects is $3.25 million, with $487,000 being spent during the 1988–89 fiscal year. After Table 2 are brief descriptions of the project objectives, scope of work and progress to date.

Table 2

MEND Research Projects

Project Number	Project	Budget (in 000s)
1.11.1	AMD From Waste Rock – Literature Review	$ 50.0
1.15.1	AMD From Open Pits – Equity Silver	$ 55.0
1.16.1	Evaluation of Prediction Techniques	$ 70.0 *
1.17.1	Hydrogeological Investigation at Waite Amulet	$ 235.0
1.21.1	Modelling of Reactive Tailings	$ 100.0
2.11.1	Evaluation of Existing Underwater Disposal Sites	$ 210.0
2.13.1	Flooding of Tailings Sites – Quirke Lake	$ 835.0
2.14.2	Vegetative Wetlands Over Tailings – Falconbridge	$ 100.0 *
2.21.1	Dry Covers for Tailings	$ 230.0
2.22.1	Assessment of Hardpan	$ 150.0
2.23.1	Documentation of Disposal Methods for Tailings and Waste Rock	$ 75.0 *
2.24.1	Vegetation Manual	$ 35.0 *
2.31.1	Dry Covers on Waste Rock – Heath Steele	$ 400.0
2.32.1	Blending and Segregation of Waste Rock	$ 140.0
2.35.1	Dry Covers on Waste Rock – Westmin	$ 200.0
3.11.1	Treatment of Acidic Seepages Employing Wetland Ecology and Microbiology	$ 230.0
3.12.1	Assessment of Existing Natural Wetlands Affected by Low pH and Low Metal Contaminated Seeps	$ 40.0
4.1.1	Field Methods Manual – Tailings	$ 20.0 *
4.3.1	Standard Reference Materials	$ 50.0
5.5.1	Research Program	$ 25.0 *
Total		**$3 250.0**

* Project complete.

Project 1.11.1 – AMD From Waste Rock Literature Review

The process of acid generation from tailings is reasonably well understood compared with the process from waste rock. Important differences between the two include oxygen and water transport and geochemical reaction rates. These differences will be reflected in prediction techniques, both chemical techniques and models, and in prevention and control strategies. This study will determine the state of understanding of acid generation from waste rock.

Project 1.15.1 – AMD From Open Pits – Equity Silver

This project will identify and quantify the relative contributions of AMD sources in open pits, develop empirical relationships, and develop, calibrate and verify models of the entire system. The Equity Silver Mine in British Columbia will conduct the project and the BC AMD Task Force (BC 2.5) will manage it.

Project 1.16.1 – Evaluation of Prediction Techniques

This project evaluated techniques for predicting the potential for tailings and waste rock to produce contaminated runoff and seepage. The initial phase of the project involved comparing 10 techniques using 9 tailings and 3 waste rock samples. Results were then compared with field data on drainage quality for the test materials. The final report was presented in June 1989.

Project 1.17.1 – Hydrogeological Investigation at Waite Amulet

This six-year project (1985–90) has been designed to develop a better understanding of the hydrogeochemical processes and changes that occur in an acid-generating tailings area. The results of the field study will also provide data to verify and calibrate predictive models for reactive tailings (Project 1.21).

Extensive site monitoring has shown that sulphide oxidation (since 1962) is limited to less than 1 m in depth. The regulating factor is apparently the penetration of gaseous oxygen with depth. Low pH values and high heavy metal concentrations have been observed in the porewater of the vadose zone. Buffering reactions in the saturated zone, however, neutralize the acidic porewater and precipitate heavy metals. Surface runoff from the vegetated tailings contain low concentrations of metals, while seepage from the toe of the tailings dam exhibits high metal concentrations and pH consistently below 3.0.

Mineralogical analyses have shown that an end-product of the chemical and biological reactions is elemental sulphur.

Project 1.21.1 – Modelling of Reactive Tailings

A comprehensive, verified model for predicting acid generation and contaminant migration in tailings is needed to:

- assist in the design of new and existing tailings disposal facilities;
- further the understanding of acid generation;
- identify key parameters for field investigation; and
- indicate new research areas.

This project builds on the Reactive Acid Tailings Assessment Program for Base Metal Tailings (RATAP.BMT) developed by CANMET. Phases a and b will be undertaken in 1989–90:

1.21.1(a): Evaluating RATAP.BMT capabilities, including calibration of the model using Waite Amulet field data, and assessing the concepts on which the model is based.

1.21.2(b): Workshop on modelling to compile different concepts for modelling reactive tailings and perceived requirements for a model, before any extensive new model development begins.

Project 2.11.1 – Evaluation of Existing Underwater Disposal Sites

It is believed that covering waste rock and tailings sites with water will minimize the transport of oxygen and therefore limit or even totally prevent acid generation. This project should establish the validity of this belief by evaluating representative existing sites. It will examine the effects when in-lake disposal systems are used and recommend criteria for the safe underwater disposal of tailings. Initially all known sites in Canada will be documented and water quality results obtained from existing sources (BC 2.3). Field surveys were done on 4 sites during the summer of 1989.

Project 2.13.1 – Flooding of Tailings Sites – Quirke Lake

Storing deposited tailings underwater in a tailings structure may be attractive if a relatively shallow water depth is sufficient to control oxidation, taking into consideration the risk of solar and wind mixing, seasonal changes in water depths, etc. Construction of a test site began in spring 1989 to evaluate the feasibility of the method.

Project 2.14.2 – Vegetative Wetlands Over Tailings – Falconbridge

The objective of this project was to develop a technique for reclaiming high iron sulphur tailings by establishing a marshland or perched water table above the tailings. The project began in 1986, when nine test cells were constructed on Falconbridge pyrrhotite tailings. One test cell was used as a control and the other eight were used to test various amendments on top of the tails, including marshland (four variations), water cover, wood chips, gravel and sewage sludge. Very promising results have been observed when a water cover could be maintained. Materials that retain moisture when the water cover was lost continued to provide reduced acid generation. The final project report was presented in April 1989.

Project 2.21.1 – Dry Covers for Tailings

This project will develop methods for testing, designing, placing and evaluating engineered dry covers to control acid generation and contaminant discharge from tailings and waste rock. Various dry covers such as clay, soils, till, polymer and synthetic membranes and cementitious materials will be evaluated for their effectiveness in controlling oxygen penetration and water percolation rates.

The first two phases of the project (2.21.1 and 2.21.2) began in January 1989. Phase 1 involves designing and testing a laboratory apparatus for evaluating covers, and the cover evaluations themselves. The results of Phase 1 will be used in Phase 2 to model oxygen penetration through the covers. The project is scheduled for completion during 1990.

Project 2.22.1 – Assessment of Hardpan

This project will characterize the mineralogy of hardpan and assess whether it can be stabilized and used as an oxygen barrier. Mineralogical studies are now being carried out on core samples from four Manitoba sites.

Project 2.23.1 – Documentation of Disposal Methods for Tailings "Draft Acid Rock Drainage Technical Guide"

A comprehensive manual has been prepared which provides guidance on how to manage wastes likely to generate acidic drainage. Techniques are discussed with respect to the handling of waste rock, tailings, open pits and underground workings. Current approaches in other parts of Canada and the world are described in the manual. The manual has been prepared as a "how to" document and is being distributed in loose leaf binder format so that it can be easily updated in the future. The report is available from the Queen's Printer in British Columbia and CANMET.

Project 2.24.1 – Vegetation Manual

During the 1970s and 1980s, mining companies and government agencies have developed and demonstrated very successful techniques for vegetating reactive tailings. MEND has requested that this technology be summarized in a state-of-the-art manual. A contract was awarded to Proctor and Redfern during the summer of 1988. The project was scheduled to be completed March 31, 1989.

Project 2.31.1 – Dry Covers on Waste Rock – Heath Steele

AMD generation in waste rock poses a much different problem than tailings because of the huge difference in the size of the waste materials. Waste rock piles contain large voids that allow water and air to freely penetrate virtually the entire pile. These conditions accelerate bacterial and chemical reactions. The reactions are so powerful that elevated temperatures (45 to 50°C) and convection currents (chimney effects) have been observed. Thus, a separate project was begun in 1988 to examine the effectiveness of different engineered covers on waste rock oxidation rates.

Heath Steele was selected for this project because the site has about 20 small waste piles. Five of the piles will be used for the study. Four of the waste piles were contoured and instrumented during the fall of 1988. Baseline monitoring will continue until the spring of 1990, when covers will be placed on the piles. The fifth pile is being moved onto an impermeable liner and instrumented during the spring of 1989. The fifth pile will allow mass balance calculations to be performed.

Project 2.32.1 – Blending and Segregation of Waste Rock – Kutcho Creek

This project has been designed to determine the effectiveness of blending or segregating acid generating waste with alkaline waste. Results of the project will be used as a model for future AMD planning by industry. Both laboratory columns and field lysimeters will be used during the course of the three year project.

Project 2.35.1 – Dry Covers on Waste Rock – Westmin

The BC AMD Task Force began this project in 1988. It was accepted as a MEND project in 1989. The objective of the project differs from the of Heath Steele project in that the former project involves the stabilization of treatment plant metal hydroxide sludges, followed by the use of these stabilized sludges as a waste rock sealant.

To date the project team has:

- determined the chemical characteristics of site leachate waters, waste rock and treatment sludges;
- completed column tests and tested various bactericides;
- conducted sludge stability tests;
- constructed six waste rock test dumps and completed baseline monitoring; and
- covered four of the waste rock dumps with stabilized treatment sludges.

Monitoring of the dumps is continuing.

Project 3.11.1 – Treatment of Acidic Seepages Employing a Combination of Wetland Ecology and Microbiology

This project follows up studies conducted over the past three years at Inco and Denison, which have shown microbially induced increases in pH from 2.5 to 5.5 over two years, including during the winter. The project will examine the role of sulphate and iron-reducing bacteria in amended ecosystems, and will determine nutrient requirements, sulphate and iron reduction rates and organic matter degradation.

Project 3.12.1 – Assessment of Existing Natural Wetlands Affected by Low pH and Low Metal Contaminated Seeps

The ability of wetlands to cope with relatively low loadings of iron, magnesium and pH has been well documented, particularly in the U.S. coal areas. The practicality of treating low pH, heavy metal contaminated, seeps from reactive tailings and waste rock is uncertain, however, and existing wetlands will be examined to determine their viability as passive treatment systems.

Project 4.1.1 – Field Methods Manual – Tailings

This project put together a field methods manual to provide guidance in the planning, conducting and assessment of sampling and monitoring of tailings projects. The guide will help ensure that sound and comparable techniques are used in sampling and monitoring. The project began in 1988, and the manual has been available since spring 1989.

Project 4.3.1 – Standard Reference Materials

This project will establish reference materials on tailings and waste rock that can be used as standards for analysis. CANMET, through the Canadian Certified Reference Materials Program (CCRMP), has prepared four different reference materials. The four samples were prepared from tailings shipped to CANMET from Inco, Falconbridge and Noranda. Analyses of 19 major and minor elements were carried out by 17 laboratories on 6 replicates for each of the four standards during 1988. Computer data entry has been completed, and computer plots of the data have been generated. Statistical outlier analyses are now being done in preparation for material certification, which was completed in the spring of 1989.

British Columbia Acid Mine Drainage Task Force

The second group in Canada coordinating research into the problem of acid mine drainage is the BC AMD Task Force, established in March 1987 by the B.C. Mining Association, mining companies, and provincial and federal governments. The purpose of the task force is to focus on, and solve special concerns of, AMD for British Columbia. These include predicting and preventing AMD from all mining wastes and the control of AMD from waste rock.

The comprehensive review and approval system (Mine Development Review Process) for new mine projects in British Columbia focuses on predicting and preventing AMD. All proposed mines are expected to conduct thorough studies to demonstrate that AMD can be addressed by technically sound and economically affordable means before mine construction. Laboratory techniques to predict AMD were developed in British Columbia in the early 1970s and have been used extensively and further refined since then. Considerable uncertainty remains, however, about the accuracy of prediction techniques and the effectiveness of proposed prevention plans.

The province contains about 72 million tonnes of acid-generating tailings, or about 4% of the total in Canada. However, about 250 million tonnes of acid-generating waste rock or 80% of the Canadian total, is increasing by about 25 million tonnes per year. As such, British Columbia has a disproportionate amount of acid-generating waste rock, and therefore the mining industry and regulatory agencies have a great interest in abating AMD from that waste type.

Like MEND, the BC AMD Task Force is divided into steering and technical committees. The technical committee is divided into three subcommittees: prediction and prevention, treatment and control, and environmental monitoring.

The goals of these subcommittees are:

- Prediction and Prevention: to review and evaluate prediction and prevention techniques;
- Treatment and Control: to find cost-effective methods to treat and control AMD; and
- Environmental Monitoring: to establish protocols for environmental monitoring of AMD.

The first activity of the task force was to define the state-of-the-art of AMD prevention technology in British Columbia. A two-part, 24-page questionnaire (5 pages for Part I and 19 pages for Part II) was prepared. Part I requested quantitative information on the type and amount of wastes produced, climate, geology, AMD potential testing, and physical characteristics of the mine wastes (water content, porosity and particle site). Part II posed general information and data availability questions on mine operations, drainage quality, history of AMD, prevention of AMD, treatment and control techniques and environmental monitoring. In September 1987, Part I was sent to 94 B.C. mining companies and Part II to the 14 companies known to have an AMD problem. In February 1988, the responses were collated and sent to a consultant (Steffen, Robertson and Kirsten Ltd. - SRK) for analysis (SRK, 1988).

SRK found that the response level to the questionnaire was reasonably good: 62% for Part I (65% for metal mines and 54% for coal mines) and 71% for Part II (77% for metal mines and 0% for coal mines - only one coal mine was canvassed for Part II). Results of the questionnaire were stored on the database software system DBase III Plus. This system will make knowledge on AMD accessible and will allow it

to be maintained and upgraded as more information on AMD in British Columbia is obtained. The database will be a source of information for researchers studying the AMD problem.

Originally, the database was supposed to conduct detailed analyses and statistical correlations of information. However, key information was missing in the responses to the questionnaire, particularly on AMD potential testing and drainage quality. The consultants made only simple statements about the nature and abatement of AMD in the province. Further analysis of the database may be possible after it is upgraded with more data.

SRK made some recommendations for the BC AMD Task Force, based on SRK's analysis of the responses to the questionnaires.

1. The emphasis of the task force should be on the abatement of AMD, not fundamental research.
2. The task force should demonstrate the best techniques to abate AMD.
3. Research should be coordinated by a small group, possibly a research unit at the University of British Columbia.
4. The task force should aim for answers within three years.
5. Research done elsewhere in the world should be integrated into the B.C. program.
6. The B.C. program should be reviewed by, and integrated into, the national MEND program.
7. When possible, research programs should "piggy-back" existing studies conducted by companies and government agencies, to conserve resources.

The technical and steering committees of the task force generally accepted these recommendations, although the research program will probably run five years, rather than three years. It is hoped that a research chair for AMD will be established at the University of British Columbia soon.

SRK also recommended some general and specific research projects. These projects were revised by the technical committee in April 1988 and a complete research program was developed (see Table 3). This $3 million program was submitted to the provincial government for approval and consideration for funding. To date, most of the provincial funding has been through the B.C.-Canada Mineral Development Agreement. Like MEND, the intent is to share the cost of AMD research, possibly equally, between government and the mining industry.

Several projects began in 1988-89, as Table 3 shows. Key reports that should soon be available include the draft technology guide for abatement of AMD (BC 1.1) and a literature survey of underwater disposal of mining wastes to prevent AMD (BC 2.3).

Planned projects for 1989-90 are in Table 4. Many of these projects have been integrated into MEND, so that the research results are shared across Canada, and to ensure that the B.C. program is not unnecessarily duplicated elsewhere in the country.

Table 3
BC AMD Task Force – Proposed Projects

General	
Coordination of program and maintenance of database	BC 1.0*
Draft technology guide for abatement of AMD	BC 1.1*
Prediction and Prevention	
Characterization of AMD potential in tailings	BC 2.1
Evaluation of prediction techniques and modelling	BC 2.2
Effect of underwater disposal in preventing AMD	BC 2.3*
Evaluation of subaerial tailings disposal system	BC 2.4*
Prediction for open pits	BC 2.5
Blending and segregation of waste rock – Kutcho Creek	BC 2.6*
Treatment and Control	
Evaluation of waste dump hydrogeochemistry	BC 3.1*
Effect of bioleaching at Gibraltar waste dump	BC 3.2
Evaluation of cover for Mount Washington waste dump	BC 3.3*
Evaluation of waste rock treatment – Westmin	BC 3.4*
Evaluation of wetland treatment	BC 3.5
Monitoring	
Optimization of sampling frequency	BC 4.1
Value of biological monitoring techniques	BC 4.2
Examination of sediment toxicity and remobilization	BC 4.3

* Project initiated in 1988–89.

Table 4
1989–90 BC AMD Task Force Projects

Prediction and Prevention	
Underwater Disposal of Wastes	BC 2.3
– Field investigations at selected sites	
Investigations of Subaerial Tailings Disposal	BC 2.4
– Completion of report and further field work at Westmin	
Prediction Open Pits	BC 2.5
– Development of model for Equity Silver pit	
Blending and Segregation	BC 2.6
– Continuation of laboratory studies and initiation of field studies at Kutcho Creek	
Treatment and Control	
Waste Dump Investigation and Analysis	BC 3.1
– Field studies at BHP Utah dump	
Gibraltar Model	BC 3.2
– Laboratory study of dump solids from bioleach operation	
Mount Washington Cover and Study	BC 3.3
– Field evaluation of till cover	
Evaluation of Waste Rock Treatment at Westmin	BC 3.4
– Continuation of field studies of cementitious cover	
Evaluation of Natural Wetland for Metal Removal	BC 3.5.1
– Equity Silver	
Evaluation of Constructed Wetland for Metal Removal	BC 3.5.2
– Noranda Bell	
Monitoring	
Sampling Optimization/Frequency	BC 4.1
– Examination of databases for selected mines	
Biological Monitoring Literature Review	BC 4.2
Biological Monitoring Apparatus Development	BC 4.3
– Development of biological trough equipment	
Sediment Monitoring Literature Review	BC 4.4

Conclusion

Economic and environmentally acceptable solutions to AMD are urgently needed. The Canadian mining industry and the federal and provincial governments have responded to this need by defining research programs and establishing cooperative methods for conducting research. MEND and the BC AMD Task Force now have about $2 million of research under way, and have proposed an additional $2 million for initiation in 1989. Future environmental requirements and reclamation practices at many Canadian mines will be determined by the results of these and future research programs.

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Microbial Degradation of Ethylene Glycol Using a Rotating Biological Contactor

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Abstract

Eleven bacterial isolates, belonging to four genera capable of growing on ethylene glycol as a sole carbon source, were isolated from two locations. The optimum conditions for ethylene glycol degradation in shake flask cultures were pH 5.0, ethylene glycol concentrations of 4 to 6 g/L, and a temperature range of 20 to 31°C. The minimum level of nutrients for a satisfactory rate of ethylene glycol degradation had the following composition in g/L: $MgSO_4$, 0.05; KH_2PO_4, 0.5; $FeCl_3$, 0.001; urea, 0.4; ethylene glycol, 3.5. The limiting factor for ethylene glycol degradation using the minimal medium was nitrogen. The organisms on the rotating biological contactor are capable of degrading 70 to 80% of the ethylene glycol at temperatures of 20 to 25°C with a retention time of 5 h and degrade 85 to 90% at temperatures of 10 to 12°C in 7.5 h (initial ethylene glycol concentration: 3.5 g/L).

Résumé

Onze isolats bactériens, appartenant à quatre genres capables de croître sur l'éthylène glycol, en l'utilisant comme seule source de carbone, ont été obtenus de deux endroits. Les conditions optimales pour la dégradation de l'éthylène glycol dans des cultures de flacons agitateurs ont été un pH de 5,0m, des concentrations d'éthylène glycol de 4 à 6 g/L et une température variant entre 20 et 31° C. Le niveau minimal des nutriments pour obtenir un taux satisfaisant de dégradation de l'éthylène glycol a été en g/L comme suit : $MgSO_4$, 0,05; KH_2PO_4, 0,5; $FeCl_3$, 0,001; urée, 0,4; éthylène glycol, 3,5. Le facteur limite de la dégradation de l'éthylène glycol, en utilisant le milieu minimal, a été l'azote. Les organismes sur le contacteur biologique rotatif sont capables de dégrader de 70 à 80% de l'éthylène glycol aux températures de 20 à 25° C pendant un temps de séjour de 5 h et d'en dégrader de 85 à 90% aux températures de 10 à 12° C pendant 7,5 h (concentration initiale de l'éthylène glycol: 3,5 g/L).

Introduction

Aircraft deicing is done with a mixture of ethylene glycol and water, generally with a 50/50 (v/v) mixture. Ethylene glycol, diethylene glycol and propylene glycol have been used for aircraft deicing, although in Canada ethylene glycol is the most frequently used deicing agent. Between 1984 and 1987, the quantity of deicing agent used at major Canadian airports varied from 97,000 L/yr (50% ethylene glycol) at the Winnipeg International Airport to 1,179,797 L/yr (50% ethylene glycol) at the Lester B. Pearson International Airport in Toronto. Glycols have a relatively low toxicity, but do affect aquatic systems because of their high biochemical oxygen demand. Thus, it is necessary to treat glycol-containing runoff from airports before the water is discharged into the environment. Numerous microorganisms are capable of degrading glycols (Child and Willetts, 1978; Cox, 1978; Dwyer and Tiedje, 1983, 1986; Watson and Jones, 1977). Rotating biological contactors (RBCs) have been used for treating municipal and industrial wastes, particularly those from the dairy and food processing industries (Hansford *et al.*, 1978).

This study will develop an economic process using an RBC for the biodegradation of airport runoff containing urea, ethylene glycol and/or propylene glycol.

Materials and Methods

Isolation of Ethylene Glycol Degrading Bacteria

Samples for the isolation of ethylene glycol degrading bacteria were obtained from two sources:

- an aerobic lagoon at the Calgary International Airport that is used for treating deicing fluids in airport runoff; and
- soil samples from several locations adjacent to taxiways alpha and bravo at the Ottawa International Airport.

Either 5 mL of water (Calgary samples) or 5 g of the various soil samples were placed in 100 mL of medium (medium #1) and placed in a rotary shaker (200 rpm at 30°C.). After one week, bacteria were isolated by streaking a sample of the cultures onto ethylene glycol agar plates.

The bacterial isolates were characterized by the following tests: oxidase reaction, Gram's stain, and the series of tests used in the Oxiferm and Enterotube II tubes (Hoffman-LaRoche Ltd.). Ethylene glycol was analyzed by oxidation of the glycol to formaldehyde followed by reaction of the aldehyde with 3-methylbenzothiazol-2-1 hydrazone hydrochloride (Evans and Dennis, 1978).

Shake Flask Studies

The effect of pH on growth and ethylene glycol degradation was determined in shake flask cultures (see Table 1). An inoculum from the RBC was added to flasks containing modified medium #2 (3.65 g ethylene glycol/L) at five different pH values. Reagent grade ethylene glycol was used for the shake flask studies. At various intervals, the bacterial growth was determined by measuring the optical density at 630 nm, and when the experiment was finished (maximum growth), the pH and ethylene glycol concentrations were determined.

The effect of ethylene glycol concentration on the degradation rate was determined in shake flask cultures using medium #3 adjusted to pH 5.5 (see Table 2).

Shake flask studies were done to determine which nutrients could be eliminated from the medium. The medium constituents and corresponding ethylene glycol degradation rates are listed in Table 3. On the basis of these results the yeast extract was eliminated from the medium entering the RBC (medium #4). A series of shake flask studies was then completed in which the effect of temperature on the rate of ethylene glycol degradation was evaluated at three glycol concentrations (see Table 4).

Shake flask studies were also carried out to determine the minimum acceptable nutrient concentrations needed for maximum ethylene glycol degradation. The various media formulations are listed in tables 5 and 6 and the corresponding glycol degradation data are listed in Table 7.

Rotating Biological Contactor Studies

The RBC used in this study has a retention volume of 30 L and four chambers containing 56 discs of 9.7 m^2 surface area. The RBC was inoculated with 2200 mL of a mixed culture containing 11 bacterial isolates obtained from the two sampling sites. Sufficient medium #2 was added to the RBC to bring the volume up to 30 L. Deicing fluid (Union Carbide UCAR D: 50% ethylene glycol) was used as the ethylene glycol source. The RBC was run with no medium addition for four days, then medium #2 was pumped through the RBC at a rate of 1.8 L/h (retention time = 16.7 h). After three weeks, the medium entering the RBC was changed to medium #3 to reflect the expected nitrogen concentrations observed in the airport runoff.

Room-Temperature Studies

The RBC was run at 23°C, with retention times ranging from 5 to 10 h using medium #4. The ethylene glycol concentrations of the influent, effluent and the four chambers of the RBC were determined every 48 h. The data are listed in tables 8 to 11.

Low-Temperature Studies

The RBC was run at 12°C and 10°C, using a retention time of 10.7 h, which was later reduced to 7.5 h. The ethylene glycol concentrations of the influent, effluent and the four chambers of the RBC were determined every 72 h. The data are listed in Table 12.

Results and Discussion

The following bacteria were isolated from the enrichment cultures: *Enterobacter agglomerans*, *Acinetobacter* sp., *Pseudomonas cepacia*, *Pseudomonas fluorescens*, *Pseudomonas putida*, and an *Alcaligenes* sp.

Shake Flask Studies

The optimal pH for growth and ethylene glycol degradation was 5.0 (see Table 1).

The optimal concentration range for ethylene glycol degradation is between 4 and 6 g/L (see Table 2). At the lower concentration, all of the glycol had been degraded by the time the flasks were sampled. Thus, the calculated degradation rates at the lowest concentrations may underestimate the actual rates.

Eliminating yeast extract from the growth medium increased the ethylene glycol degradation rate. The predominant microorganism in the RBC, *Pseudomonas putida*, does not need the additional growth factors in yeast extract. The yeast extract may actually inhibit the degradation of ethylene glycol since the bacteria may use the yeast extract as a nutrient in preference to the glycol. Removing either $FeCl_3$ or $MgSO_4$ from the medium appeared to decrease the rate of ethylene glycol degradation. The optimal temperature for ethylene glycol degradation, from the shake flask studies, appears to be between 20 and 31°C, although appreciable degradation was observed at 10°C and 37°C (see Table 4).

The rate of ethylene glycol degradation decreased slightly at magnesium sulphate concentrations below 0.05 g/L (see tables 5 and 7, media G1–G3). The glycol degradation rate was also found to decrease at potassium phosphate concentrations below 0.5 g/L (see tables 5 and 7, media H1–H3). Decreasing the iron concentration had little effect on the degradation of ethylene glycol (see tables 6 and 7, media J1–J3). However the glycol degradation rate was greatly affected by the nitrogen concentration of the medium (see tables 6 and 7, media N1–N4). It appears that nitrogen is the limiting nutrient in the medium now used.

The concentrations of nutrients used in the shake flask studies were somewhat different from that used in the RBC. The ethylene glycol concentration was 5 g/L in the shake flask cultures and 3.5 g/L in the RBC. A slightly higher phosphate concentration was also used in the nitrogen test shake flask studies. Although the shake flask studies cannot be used directly to predict ethylene glycol degradation rates in an RBC, comparative degradation rates in the shake flask cultures are still valid. Medium #4 was found to be the most satisfactory one to date.

Rotating Biological Contactor Studies

Colonization of the RBC discs by the bacteria was very slow for the first two weeks, but some growth was observed by the third week. During the third week a strong ammonia smell was noticed. Hydrolysis of urea in the RBC would result in an increase in pH and ammonium ion concentration, which would cause the evolution of ammonia. The influent medium was changed to correct this problem. The urea concentration was decreased by a factor of 10 and the pH was lowered to 5.5 (medium #3).

The degradation rate of ethylene glycol in the RBC was initially fairly low (see Table 8), but as the biomass continued to accumulate the degradation rate increased. The predominant organism present in the RBC has been identified as *Pseudomonas putida*. This bacterial species is considered to be non–pathogenic and should present no handling problems on an industrial scale.

Initially 40% of the added glycol was degraded (see Table 8) in the RBC under the following operating conditions: initial ethylene glycol concentration 5.5 g/L at 18°C, and a retention time of 18.4 h. After more biomass had accumulated on the discs and the medium constituents had been modified, the RBC was capable of degrading 70 to 80% of the added glycol under the following conditions: 3.5 g/L ethylene glycol at 23°C, and a retention time of 5 h. When the optimal conditions for the degradation of the ethylene glycol had been established, the performance of the RBC at lower temperatures was evaluated. In contrast to the shake flask studies, the degradation of ethylene glycol occurred more rapidly at lower temperatures (see Table 12). Almost 85 to 90% of the added glycol was degraded at 10 to 12°C by the RBC (see Table 12). At lower temperatures bacterial membranes are less fluid (containing a lower percentage of unsaturated fatty acids), and thus may not be as sensitive to the solvent effects of ethylene glycol.

Conclusions

A process for treating airport runoff containing ethylene glycol using an RBC has been developed. To obtain satisfactory ethylene glycol degradation rates, the runoff must be amended with a low level of nutrients ($MgSO_4$ and $(NH_4)_2HPO_4$) and the temperature in the RBC should be maintained at 10°C or above. A laboratory-scale RBC (30 L capacity) was found to degrade 336 g of ethylene glycol per day. Assuming that the process can be scaled up in a linear fashion, a treatment system for the Halifax International Airport (annual glycol usage: 200,000 L/yr) would need three industrial-scale RBCs (63,000 L capacity). However, to scale up a system for treating airport runoff, a pilot study must be set up at one of the airports. Sufficient storage capacity to deal with large variations in flow and ethylene glycol concentrations will be the major engineering problem in designing a treatment system. Compared with other treatment systems, the RBC has many advantages. For example, an RBC would require less space and degrade ethylene glycol more efficiently than an aerated lagoon.

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Various Media Used to Study the Degradation of Ethylene Glycol

Medium	Constituents	Concentration (g/L)	
#1	$MgSO_4$	0.1	(pH 7.7)
	Yeast extract	0.05	
	K_2HPO_4	1.0	
	Urea	0.5	
	Ethylene glycol	2.0	
#2	$MgSO_4$	0.1	(pH 7.7)
	Yeast extract	0.05	
	K_2HPO_4	1.0	
	Urea	2.0	
	$FeCl_3$	0.05	
	Ethylene glycol	10.0	
#3	$MgSO_4$	0.1	(pH 5.5)
	Yeast extract	0.05	
	KH_2PO_4	1.0	
	Urea	0.2	
	Ethylene glycol	5.0	
	$FeCl_3$	0.025	
#4	$MgSO_4$	0.05	(pH 5.5)
	KH_2PO_4	0.5	
	Urea	0.4	
	Ethylene glycol	3.5	
	$FeCl_3$	0.001	

Table 1
Effect of Initial pH on Bacterial Growth and Ethylene Glycol Degradation

Initial pH	Final pH	Bacterial Growth (O.D. at 630 nm) Incubation Time (h)				Ethylene Glycol (g/L*)
		0	19	43	115	
5.0	7.71	0.27	0.79	1.02	2.34	0.38
6.0	5.58	0.22	0.69	0.82	0.93	0.78
7.0	4.90	0.25	0.59	0.68	0.85	1.13
8.0	8.15	0.22	0.55	0.52	0.47	1.70
9.0	8.41	0.26	0.62	0.52	0.37	1.93

*Initial ethylene glycol 3.6 g/L.

Table 2
Effect of Concentration on the Degradation of Ethylene Glycol
(Analyses Performed after 41 h in Shake Flask Culture)

Initial Ethylene Glycol Concentration	Ethylene Glycol Degradation Rate (mg/L/h)	% of Initial Ethylene Glycol Degraded
1.75*	43	100
2.70	55	84
3.75	73	80
5.80	91	71
6.60	89	60
7.75	75	44

*Initial values are based on analyses of control flasks with no bacteria.

Table 3
Effect of Medium Constituents on the Degradation of Ethylene Glycol in Shake Flasks

Treatment	Ethylene Glycol Concentration, g/L			
	Time (h)			
	0	24	48	72
Complete medium	5.1	4.7	4.4	3.2
Medium A	4.0	3.9	3.0	2.1
Medium B	4.6	4.3	3.6	3.9
Medium C	4.3	4.1	3.3	3.6

Medium Constituents	g/L	Medium Composition		
		A	B	C
$MgSO_4$	0.1			no $MgSO_4$
Yeast Extract	0.05	no yeast extract		
KH_2PO_4	1.0			
Urea	0.2			
Ethylene glycol	5.0			
$FeCl_3$	0.0125		no $FeCl_3$	

Table 4
Effect of Temperature on the Degradation of Ethylene Glycol in Shake Flasks

Time (h)	10°C			20°C			31°C			37°C		
(Initial Conc.)	(3)	(5)	(8)	(3)	(5)	(8)	(3)	(5)	(8)	(3)	(5)	(8)
0	3.6	5.4	8.8	3.4	5.1	6.8	3.2	5.4	8.1	3.5	5.3	9.4
24	–	–	–	3.2	4.7	6.2	2.9	4.9	7.3	3.4	5.0	9.1
48	–	–	–	2.0	4.4	6.6	–	–	–	–	–	–
72	3.0	5.6	8.1	–	3.2	–	–	–	–	–	–	–
120	0.4	1.6	5.4	0	–	4.4	–	–	–	–	–	–

Table 5
Various Media Compositions Used to Determine the Minimum Nutrient Requirements for Ethylene Glycol Degradation

Constituents	Additions (g/L)					
	G1	G2	G3	H1	H2	H3
$MgSO_4$	0.05	0.03	0.01	0.05	0.05	0.05
KH_2PO_4	0.5	0.5	0.5	0.5	0.3	0.1
$FeCl_3$	0.01	0.01	0.01	0.01	0.01	0.01
Urea	0.2	0.2	0.2	0.2	0.2	0.2
Ethylene glycol	5.0	5.0	5.0	5.0	5.0	5.0

Table 6
Various Media Compositions Used to Determine the Minimum Nutrient Requirements for Ethylene Glycol Degradation

Constituents	Additions (g/L)						
	J1	J2	J3	N1	N2	N3	N4
$MgSO_4$	0.05	0.05	0.05	0.05	0.05	0.05	0.05
KH_2PO_4	0.5	0.5	0.5	1.0	1.0	1.0	1.0
$FeCl_3$	0.01	0.005	0.001	0.009	0.009	0.009	0.009
Urea	0.2	0.2	0.2	0.1	0.2	0.3	0.4
Ethylene glycol	5.0	5.0	5.0	5.0	5.0	5.0	5.0

Table 7
Degradation of Ethylene Glycol in Shake Flask Cultures Using Various Media Compositions

Medium	Ethylene Glycol Concentration (g/L)			
	Initial	24 h	48 h	72 h
G1	5.3	4.9	3.1	1.3
G2	5.3	5.4	3.7	1.6
G3	5.3	5.6	4.8	2.1
H1	5.3	5.2	4.8	1.4
H2	5.3	5.4	4.8	1.8
H3	5.3	5.6	4.7	2.5
J1	5.3	5.5	3.9	1.9
J2	5.3	5.4	4.7	2.4
J3	5.3	5.3	4.8	2.1
N1	5.1	4.9	3.7	2.4
N2	5.1	4.7	2.8	0
N3	5.1	5.0	2.8	0
N4	5.1	4.8	1.6	0

Table 8
Ethylene Glycol Concentrations at Various Locations in an RBC (September 1988)

Sample Location	Ethylene Glycol Concentration (g/L)
Influent tank	5.5
First chamber	4.7
Second chamber	4.0
Third chamber	3.5
Fourth chamber	3.2
Effluent	2.4

Table 9
Effect of Residence Time on the Degradation of Ethylene Glycol in an RBC

Chamber	Ethylene Glycol Concentration (g/L)					
	Oct. 28	Oct. 31	Nov. 1	Nov. 3	Nov. 4	Nov. 7
Influent	4.4	4.8	5.0	4.6	4.2	5.4
First	1.5	1.5	3.1	2.3	2.9	3.1
Second	0.9	1.0	2.9	2.4	2.4	2.5
Third	1.2	1.1	2.3	1.4	1.7	1.7
Fourth	0.6	0.9	2.2	1.4	1.6	1.3
Effluent	1.0	0.9	2.0	1.2	2.0	1.7
Flow rate (L/h)	1.63	1.63	2	3	4	5
Residence Time (h)	18.4	18.4	15	10	8	6

Table 10
Effect of Various Parameters on the Degradation of Ethylene Glycol in an RBC (Retention Time 6.0 h)

Chamber	Ethylene Glycol Concentration (g/L)					
	Nov. 14	Nov. 16	Nov. 18	Nov. 29	Dec. 1	Dec. 5
Influent	3.9	2.22*	2.66**	3.0***	3.57	3.6
First	3.4	0.88	2.0	2.1	1.94	2.5
Second	2.9	0.80	1.54	1.6	1.56	2.1
Third	2.2	0.63	0.53	1.4	1.02	2.1
Fourth	1.5	0.69	0.66	1.0	0.94	1.4
Effluent	1.6	0.77	–	1.3	0.78	1.1

* Feed changed to 2.5 g/L ethylene glycol.

** Feed changed to 3.5 g/L ethylene glycol.

*** Yeast extract eliminated from feed.

Table 11
Effect of Various Parameters on the Degradation of Ethylene Glycol in an REC

Chamber	Ethylene Glycol Concentration (g/L)				
	Jan. 18	Jan. 25	Feb. 3	Feb. 8	Feb. 15
Influent	3.1	3.7	2.8*	3.9	3.4
First	2.6	2.6	1.9	3.0	1.6
Second	2.4	2.1	1.4	1.9	0.9
Third	1.8	1.7	0.6	1.4	0.8
Fourth	1.7	1.4	0.9	1.0	0.7
Effluent	1.2	1.3	0	1.2	0.6
Flow rate (L/h)	6	3	3	6	4.25
Residence time (h)	5	10	10	5	7.0

* Feed changed from 0.2 g/L urea to 0.4 g/L urea.

Table 12
Ethylene Glycol Degradation at Various Temperatures in the RBC

Chamber	Ethylene Glycol Concentration (g/L)					
	Feb. 28	Mar. 3	Mar. 6	Mar. 8	Mar. 10	Mar. 13
Influent	3.4	3.1	3.1	3.2	3.3	4.1
First	1.7	2.4	2.0	2.1	2.3	3.5
Second	1.3	1.4	0.9	1.0	1.6	2.2
Third	0.7	1.0	0.6	0.7	1.1	1.4
Fourth	0.7	0.8	0.6	0.4	0.7	0.9
Effluent	0.5	0.7	0.4	0.3	0.5	0.6
Temperature (°C)	23.5	20	12	12	10	10
Flowrate (L/h)	4.0	4.2	2.8	4.0	4.0	4.0
Residence Time (h)	7.5	7.1	10.7	7.5	7.5	7.5
% Degradation	84	76	86	91	84	84

Neutralization of Acid Mine Drainage Using Microbial Processes

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Abstract

Troughs were set up in the laboratory to study microbial neutralization of acid mine drainage (AMD) using cellulosic materials as both support and carbon source for the microorganisms. Artificial AMD of pH 3.5 containing sulphate 900 ppm, iron 100 ppm, phosphate, urea, dextrose and magnesium was effectively treated: the effluent of pH higher than 7.5 showed 100% reduction of the iron content and up to 85% reduction of the sulphate content. A field test, corresponding to a 550-fold scaleup of the laboratory experiment, was initiated at the Halifax International Airport in August 1989. Three cells of 17 by 3 m containing straw and wood shavings were constructed on a sub-drain drainage ditch and inoculated with sulphate-reducing bacteria taken from the laboratory setup. Acid water of pH 3.5 and sulphate content 500 ppm was supplemented with urea, sugar and ground apatite ore. After one month, the sulphate content was reduced 30% and the pH was raised from 3.5 to 4.2.

Résumé

Des bassins ont été installés dans le laboratoire pour étudier la neutralisation microbienne des eaux de drainage acides (EDA) des mines en utilisant des matières cellulosiques comme support et comme source de carbone pour les micro-organismes. Des eaux de drainage acides démontrant un pH de 3,5 et contenant 900 ppm de sulfate, 100 ppm de fer, du phosphate, de l'urée, du dextrose et du magnésium ont été traitées; l'effluent ayant un pH dépassant 7,5 et la teneur en fer affichait une réduction de 100% et celle du sulfate jusqu'à 85%. En août 1989, on a entrepris, à l'aéroport international de Halifax, un essai sur le terrain correspondant à une mise à l'échelle de 550 fois l'expérience de laboratoire. On a construit trois cellules de 17 sur 3 m contenant de la paille et des copeaux de bois sur un drain à écoulement souterrain dans lequel on a inoculé des bactéries de réduction du sulfate provenant du laboratoire. Le pH de 3,5 et la teneur en sulfate de 500 ppm de l'eau ont été obtenus par l'ajout d'urée, de sucre et de minerai apatitique. Après un mois, la teneur en sulfate a diminué de 30% et le pH est passé de 3,5 à 4,2.

Introduction

Acid mine drainage (AMD) is an important environmental pollutant, for which no truly effective, acceptable treatment is available (Filion and Ferguson, 1989). AMD is characterized by high acidity and low pH, a high sulphate content, and the presence of dissolved metal ions such as iron or aluminum. An effective treatment is sought to generate water of low acidity and neutral pH, and to eliminate part of the sulphate, iron and other elements present. To be attractive, this process must be cheap, easy to install and maintain, and must not produce byproducts that are difficult to eliminate.

Sulphate-reducing bacteria (SRB) have been implicated in acid mitigation in freshwaters (Tuttle *et al.*, 1969a; Schindler and Turner, 1982; Herlihy and Mills, 1985; Cairns *et al.*, 1988). Herlihy *et al.* (1987) measured up to 48% removal of the sulphate present in an impoundment receiving AMD by SRB in sediments. A high percentage of the sulphide produced from sulphate was retained in the sediments as iron sulphide. Tuttle *et al.* (1969b) reported on the mitigation of acidic mine water permeating through a dam composed mainly of wood dust. Cellulose degradation of the wood dust by mixed cultures was providing nutrients and creating anaerobic conditions for the SRB. The authors proposed the biological treatment of AMD using a process based on the microbial degradation of wood dust. Microbial mitigation of AMD of pH 2.3 from base-metal tailings was also shown to occur at a site amended with straw (Cairns *et al.*, 1988).

In laboratory flask cultures, mixed populations containing SRB were able to reduce sulphate in acidic water at pH 3.0 with sawdust as the only nutrient. Pure cultures of SRB do not reduce sulphate at pH less than 5.5 (Tuttle *et al.*, 1969b). Other microbial populations are involved in the increase of pH (Jongejan, 1982; Cairns *et al.*, 1988).

Microbial mitigation of acid mine water in a continuous-flow system in the laboratory was obtained by Wakao *et al.* (1979). In 10 L fermentation bottles, mixed cultures containing SRB neutralized pH 4.0 acid mine water in the presence of wood dust and organic nutrients. However, when the acid water was fed continuously at pH 3.0, a steady-state system was not established.

In the present study, a continuous-flow pilot-scale model was developed to study the processes involved in the microbial neutralization of AMD using cellulosic material. At the same time, field tests were initiated at Halifax International Airport, and this paper presents preliminary results. A better understanding of the overall process, and the various parameters that affect it, should enable us to develop an effective and relatively inexpensive treatment process for AMD.

Materials and Methods

Setup of Pilot-Scale Model

A metal frame 6.5 ft high by 5 ft wide was used to hold four PVC and lucite troughs (4 ft long by 5 in. wide by 8 in. deep). The troughs were connected with Tygon tubing 20 mm (7/16") in diameter. Each trough was sloped so that water flowing along the length of the trough entered it at a higher elevation than it exited. Water traversed through the four troughs, one after the other, from top trough to bottom trough.

A peristaltic pump (Masterflex Model 7015-21, Cole-Parmer Instrument Co., Chicago, Illinois) was used to control the flow rate. The flow rate was initially set at 33 mL/min, and was reduced to 22 mL/min for the last two months of this study. Each trough was initially filled with straw, planer shavings, or a mixture of both. A few grams of partially degraded cellulose fiber containing slight amounts of wax, phenolic resins and alum were included in the shavings. When necessary, straw was added. All work was done at room temperature.

Artificial Acid Water

Tap water containing yeast extract and dextrose (1 g/L each) was recirculated through the troughs for the first month. The effect of various microbial nutrients were assessed by adding the following components to the artificial AMD: sodium lactate 60% solution, 0.46 mL/L; ferrous sulphate and magnesium sulphate, varying concentrations; peptone, 0.2 g/L; starch, 0.2 g/L; dextrose, 1 g/L; potassium phosphate dibasic, 0.027 g/L; and urea, 0.1 g/L. The final composition of the artificial acid water used in this study starting May 1989 was (in mg/L): $MgSO_4.7H_2O$, 1667; $FeSO_4.7H_2O$, 500; dextrose, 1000; urea, 100; and K_2HPO_4, 27. This corresponds to an iron concentration of 100 ppm, sulphate concentration of 900 ppm, and phosphorus concentration of 15 ppm. Concentrated sulphuric acid was used to adjust the pH. The pH of the artificial AMD was slowly acidified for several months. Artificial AMD at pH 3.5 was used from July 1989.

Halifax Field Tests

A shallow ditch 1 to 2 m wide containing AMD at pH 2.9 to 5.0, containing sulphate (20 to 730 ppm), iron (0.82 to 135 ppm) and aluminum (1.1 to 48 ppm) (unpublished data, 1988), was chosen as the site for both the field tests. This ditch receives drainage from the runway sub-drains.

In August 1988, two 16 m cells were installed in the ditch one after the other. Planer shavings were dumped and held in place by rocks and 5 mm plastic mesh. Cellulose fibres identical to the ones used in the pilot-scale experiment, and black mud containing SRB were incorporated into the wood shavings.

In August 1989, both cells installed the previous year were dismantled and a 550-fold scaleup of the laboratory model was constructed next to the ditch. A plywood dam was used to divert the stream into three clay-lined cells, located sequentially parallel to the ditch, each 16.8 m long by about 1 m wide. Water flowed from one cell to the other through three plastic pipes of 150 mm diameter. After treatment, water returned to the ditch downstream. Water capacity of the system was 23 L/min; excess water flowed over the dam directly into the ditch. All cells contained fresh planer shavings and partially decomposed straw as cellulosic materials in a proportion of 1:3. A total of 54 kg of Lantic sugar, 1 kg of phosphate rock (supplied by the Smelting and Fertilizer Division, Brunswick Mining and Smelting Corporation Ltd., Belledune, New Brunswick) and 6 kg of urea fertilizer (C-I-L 46-0-0) was distributed among the cells. Used straw and wood shavings from the second trough in the pilot-scale model containing various microorganisms including SRB were incorporated into the cells as a source of inoculum. After the initial startup, 40 kg of Lantic sugar and 4 kg of urea fertilizer were added weekly to the acid water as it entered the first cell.

Microorganisms

All microorganisms participating in AMD neutralization were in the materials used, or in the immediate environment of the troughs or the cells.

Chemical and Physical Analyses

For the pilot-scale testing, chemical analyses were done at CANMET. Influent samples were taken directly from the influent tank. Effluent samples were collected as the treated water exited from the bottom trough.

The pH measurements were on filtered or non-filtered samples, using a Fisher Accumet pH-meter Model 925 (Fisher Scientific, Pittsburgh, Pennsylvania). The pH-meter was calibrated with fresh buffer solutions each time.

Sulphate analysis was done by a standard gravimetric method, as described by (Gould *et al.*, 1988). The error of the method was 5% (standard methods for the examination of water and wastewater, 1980). Sulphate analysis for the last four samples was done by high-pressure liquid chromatography, after filtering samples with a 0.2 μm filter. The analysis was done on a DIONEX Model 2110i (DIONEX, Sunnyvale, California) using a "fast run" ion-exchange column for anions, and a conductibility detector. A mixture of sodium bicarbonate and sodium carbonate was used as the elution solution. The error of this method is less than 2%.

Iron analysis was by atomic absorption (AA) using a Varian AA spectrophotometer Model AA775 (upgraded to AA875) (Varian, Springvale, Australia). The samples were filtered through Whatman paper No. 42 and acidified with HCl before analysis.

Flow rate within the pilot-scale model was measured by collecting effluent in a graduated cylinder for a 5 min period, using a digital timer, and repeating once.

At the Halifax field test site, water samples were taken weekly upstream, downstream and between each cell using standard techniques. Temperature and precipitation were measured in the field. An estimate of the pH of the water was obtained on site using pH paper. Flow rate during the second field test was measured with a stopwatch by collecting water coming out of the third cell in a 1 L container. Readings were taken three to four times, and the final value given is an average. Water samples were then sent for analysis to the Laboratory of Environmental Chemistry, Division of Clinical Chemistry, Victoria General Hospital, Halifax, Nova Scotia. In 1988 samples were tested for total acidity, pH, total solids, sulphate, iron and aluminum. In 1989, NH_4 (nitrate and ammonia), PO_4 (ortho) and total organic carbon content were also determined.

Microbial Analyses

Once a month samples of straw and/or wood shavings mixed with water from the sampling site were taken at every 3 m of the test cells (four samples per cells) for the first field test, and at every one-third and two-thirds of each cell (two samples per cell) for the second field test. The samples were sent to CANMET for analysis. For the second field test, samples were a mixture of material taken at the air-water interface and at the bottom of the cell.

Total fungal counts were assessed using Sabouraud dextrose agar acidified to 3.5 with dilute H_2SO_4. Total aerobic heterotrophic bacteria were assessed on tryptic soy agar (TSA) containing cycloheximide 100µg/mL, acidified to pH 3.5. Sequential dilutions (1 mL of liquid portion of well-shaken sample in 10 mL of 0.05% of peptone) were made to obtain eight dilutions of the sample, and 0.1 mL of each dilution was plated on the appropriate medium in triplicate. The plates were incubated at 29°C for 48 h for the bacterial count, and 72 h for the fungal count. Colony counts were conducted with a Quebec colony counter.

The number of SRB was estimated using strict anaerobic techniques for *Desulfovibrio* sp. by doing sequential "deep-agar" dilutions in triplicate (Postgate, 1984). The medium is the modified medium C described by Singleton *et al.* (1988), but instead of using an anaerobic glove box, nitrogen gas was bubbled through the cysteine, phosphate and iron solutions in a sterile manner. Samples were prepared for analysis in the following manner: straw and/or wood shavings and water from the sample were transferred into a small vial containing glass beads and mixed with a small vibrator to detach organisms from the solid support. One millilitre of the liquid portion was then used for the agar dilutions. The bacteria were incubated at 35°C; counts were determined 48 h and 10 days after inoculation.

Results

Pilot-scale Model

After recirculating tap water containing nutrients for one month, SRB were shown to be present in all four troughs at a concentration greater than 1×10^5. Thereafter, "artificial AMD" was fed in continuous flow, starting at pH 5.5, which was the final pH measured for the recirculating water. For six months, influent pH was slowly dropped to pH 3.5. As early as one week after startup of the continuous-flow system using only tap water and magnesium sulphate, effluent pH higher than 6.8 was measured. However, within a month, the pH of the effluent dropped to 6.0, even though pH of the influent was still higher than 5.0. It was necessary to add nutrients such as phosphate and nitrogen (as urea) to sustain the microorganisms. Peptone, starch and dextrose were tried, one after the other, as possible stimulants for the process. Dextrose induced the greatest pH change and removal of sulphate, and so was incorporated into the acid water composition. Iron was added to influent of pH 5.0 or less. At greater pH, the ferrous iron rapidly oxidized and precipitated out of solution, greatly modifying the chemistry of the influent.

Figure 1 presents pH measurements taken from the moment the artificial acid water's final composition was determined. The first 70 days correspond to a time period where the system is still adjusting to pH changes, as the influent drops from pH 4.5 to 3.5. Even so, at all times the pH of the effluent was greater than 6.0. The amount of sulphate removed from the acid water over the same period of time is presented in Figure 2. Sulphate removal seems to be more sensitive to changes than pH, as greater variations are observed in this figure. In particular, toward day 70 the dextrose content of the influent was decreased by 90%. The amount of sulphate removed dropped from 60 to 40%, while the pH of the effluent decreased by less than one pH unit. At about 80 days, all the straw and wood shavings in the second trough were removed and taken to Halifax Airport for use as an inoculum for the second field test. These were replaced in the laboratory experiment with fresh straw. Immediately after adding the fresh straw, although dextrose concentration was still 100 mg/L, the pH of the effluent peaked at 8.0 and sulphate removal increased

from 20 to 45%. However, the positive effect of straw addition was short-lived, and dextrose concentration had to be increased to 500 mg/L. This was followed by a rise in the effluent pH, and a greater amount of sulphate was removed from the artificial acid water. A maximum of 85% sulphate removal was subsequently measured. During this time, 100% of the 100 ppm iron present in the influent was removed. A grey-black precipitate was continuously formed in the troughs.

Halifax Field Tests

The first field test, started in August 1988, was unsuccessful. Figure 3 shows pH values measured in the laboratory for water before and after passage through both wood channels. Clearly no change in pH was observed. Likewise no difference was observed for other parameters that were monitored.

Microbial counts were done four times, one month after the startup of the test, until December. Figure 4 presents results obtained for all the samples from site A1, located within the first 3 m of the first cell. Results given for the month of September correspond to a total count, including both fungi and heterotrophic bacteria. The number of cells present was low and decreased with time. The values presented in this figure for the total heterotrophic aerobic bacteria should be interpreted as less than 300 cells/mL; the number of colonies counted were too low to give a closer estimate that could be considered statistically correct. A medium containing a fungal inhibitor, cycloheximide, had to be used for bacterial counts because fungi outnumbered and outgrew bacteria on a regular medium. Most fungi present were common soil fungi, such as *Rhizopus* or *Penicillium*. Yeasts such as *Candida* and *Cryptococcus* were also found. Results obtained for the other sampling sites were very similar to those obtained for A1.

The wood shavings in the samples showed no sign of degradation, and the water was slightly orange, characteristic of oxidized iron. Thus no attempt was made to isolate SRB from these samples, as these microorganisms need very reduced conditions for growth.

A total of 152 mm of precipitation was monitored in the 48 h preceding water sampling. A month after startup, 10 to 15% of the water was bypassing the first channel, and 15 to 20% was bypassing the second, flooding the flat area surrounding the ditch. Two months later, up to 60% of the water was bypassing both channels. The temperature at the field site was 25°C at startup, decreased to less than 20°C during September, less than 10°C during October, and less than 5°C after mid-November. In December the stream froze.

The second field test began in August 1989. The three clay cells and the dam were built to avoid flooding.

Figure 5 shows pH values measured in the laboratory during the first six weeks of operation. Although no pronounced change has been recorded in the pH, other parameters measured are promising. Total acidity decreased as water flowed from cell to cell, starting on day one when an 8% decrease in acidity was obtained. On day 42, a 69% acidity decrease was reported. Sulphate removal first occurred three weeks after startup. Five weeks after startup a slight sulphide smell was noticed during water sampling. After six weeks (day 42 on Figure 5) 52% of the sulphate in the acid water was removed during passage through all three cells: 17% in the first cell, 22% in the second cell and 13% in the third cell. Analysis of samples taken on day 42 also showed that 93% of the aluminum present was eliminated; no decrease in aluminum content had been registered previously. Total organic carbon (TOC) and phosphate increased very slightly, but were still present at very low concentrations: less than 14 mg/L for TOC, and less than 0.6 mg/L for phosphate. Other parameters studied decreased or stayed constant.

Microbial counts were conducted one month after startup (see Figure 6). Samples received were dark brown, with a faint sulphide smell. Numbers shown along the x-axis in Figure 6 represent sampling sites: 1-1/3 corresponds to a site located one-third the distance downstream in the first cell; 1-2/3 corresponds to a site located two-thirds the distance downstream within the first cell. SRB were in all samples; the concentrations ranged from 1 x 10^3 cells/mL for samples for sites 1-2/3 and 3-2/3 to 2.4 x 10^5 cells/mL for sample 2-2/3. Fungi were present in varying numbers, and species were again essentially common soil microorganisms. Yeasts were also present. Essentially two types of bacterial colonies were observed on TSA: minute colonies (less than 2 mm in diameter) and small cream colonies. Observation of the bacteria from the cream colonies revealed Gram-positive cocci in clusters similar to *Staphylococcus* sp.

Flow rate of the water exiting the last cell varied from 8 to 12 L/min. Almost no precipitation was recorded during the first six weeks of operation, and the air temperature recorded varied between 13 and 20°C.

Discussion

Microbial mitigation of AMD using cellulosic material as a support and nutrient source for microorganisms is a potentially interesting treatment. The most effective treatment in widespread use today is liming. However, lime treatment plants require maintenance, are costly to operate and produce vast amounts of sludge. Biological treatment of AMD using cellulosic material would be cheap to install and would require minimal maintenance. The amount of wastes generated would be considerably less than the amount of sludge produced today; it is expected that after a few years, partly decomposed celullosic material would need to be replaced by fresh material.

A laboratory model that could simulate field conditions was needed to study the microorganisms involved and the various factors that influence them. Although Wakao *et al.* (1979) looked at the treatment of AMD in continuous flow, they used a small system that did not reproduce the physical environment of a stream. Aeration of the water being treated, in particular, was not representative of what happens in the field. Jongejan (1983) reported that pH increase in the presence of woodwaste depended more on the woodwaste length through which the water flowed, than on the wood volume. To the best of our knowledge, no other author has described a system such as ours.

Successful mitigation of AMD at pH 3.5 was recorded for more than three months in the laboratory in the presence of straw and woodwaste. Previous work using only woodwaste (unpublished results) did not permit the establishment of a self-sustaining treatment process. Wood is recalcitrant to biodegradation. Lignocellulose makes up 89 to 98% of the dry weight of wood (Colberg, 1988). Cellulose is the most abundant and the most readily degradable component of lignocellulose. However, in wood it is physically surrounded by lignin, the second most abundant and highly recalcitrant fraction of lignocellulose. Thus, wood cellulose is not readily available. If wood degradation is occurring too slowly, microorganisms that participate in the mitigation process cannot get enough nutrients for growth. Also, most of the cellulosic material is submerged in water under microaerophilic or anaerobic conditions. Although there are reports of plant lignocellulose degradation in anaerobic conditions in aquatic systems (Federle and Vistal, 1980; Benner *et al.*, 1984), the anaerobic degradation of wood has never been reported (Colberg, 1988). Since straw was shown to sustain microbial mitigation of AMD (Cairns *et al.*, 1988), it was incorporated into the troughs as a source of both readily available nutrients and cellulolytic microorganisms. The beneficial

effect of soluble nutrients from straw was seen in figures 1 and 2. However, this effect is short-lived. Straw is mainly useful as a source of cellulose that is more accessible to the microorganisms than wood. It accelerates the rate of colonization because of the cellulose-degrading organisms in the system. These cellulose-degraders are thereafter expected to attack the more recalcitrant wood shavings.

Dextrose proved to enhance the process, as reported by Tuttle *et al.* (1969a). These authors found that adding glucose to a fresh flask culture containing wood dust and acid water improved sulphate reduction at 1,000 mg/L, inhibited it at 10,000 mg/L and had no effect at 10 mg/L. In our system, the amount of dextrose has been successfully decreased to 500 mg/L. Dextrose or another simple sugar may be critical to the startup of the process. White-rot fungi, the only organisms known to totally degrade wood, need an easily accessible energy source to attack lignin, and it is thought that low molecular weight sugars as well as polysaccharides in wood serve as co-substrate (Eriksson, 1981). Until the release of sugars from wood is sufficient to sustain the organisms, another source may have to be supplied. This may explain why peptone did not give similar results to those obtained with dextrose. Initially, fermentative bacteria grow faster than the fungi and metabolize all the sugars. We eventually hope to eliminate dextrose addition.

Sulphate removal is the result of microbial activity, both in the laboratory and in the field: sulphate-reducers were present; sulphide was produced; and sulphate was continually removed from the acid water. All the iron present in AMD in the laboratory was removed during the treatment process, presumably as FeS (Tuttle *et al.*, 1969b). Mineralogical analysis of the grey-black precipitate will soon be completed. It is important to confirm that metals are being precipitated as metal sulphides. Sulphide minerals are insoluble under anaerobic conditions. As long as the cellulosic material containing the precipitate remains undisturbed, in cells or in a burial site, anaerobic conditions will prevail, and this treatment can be considered permanent. Some metals and elements are also expected to adsorb to the cellulosic material, or to the biomass growing on it. Certain fungi and algae have a great capacity for adsorbing elements such as uranium (Wood, 1984). Metals such as iron can also be chelated by microbial products such as siderophores. The model developed in the laboratory can be used to look at the elimination of various metals and elements from AMD. More important, now that we have a working process, microbiological studies can begin.

Results from the first field test were disappointing. Clearly the wood channels acted as a dam, and a new design was proposed to prevent flooding and to ensure that a maximum amount of the AMD was treated. It is also thought that poor microbial growth was caused by a lack of nutrients and by less than optimal temperatures. Although at near-freezing temperatures most microbial activities are extremely slow, sufficient microbial activity within the system could generate an excess of energy that could be released as heat, so that activity might continue throughout the cold season. It was also hoped that snow would act as insulation. All this was considered when preparing the second field test.

Results from the second field test were encouraging: acidity, aluminum and sulphate content decreased; microbial counts were 100 to 500 times higher than those recorded one month after startup of the first field test; and a large population of SRB was present. For an efficient startup, it may prove necessary to inoculate cellulosic material with microorganisms, as we did for the second field test. Adding nutrients also seemed to contribute to startup of the process, as it did with the pilot-scale model. However, the use of urea and dextrose is considered a temporary measure. The use of cheap, readily available materials that slowly release the nutrients needs to be investigated. This is essential to reduce the cost and maintenance needs of the process. Phosphate rock (ground apatite ore) has already been used. However,

because of the complex microbial and chemical interactions involved in the microbial mitigation of AMD, preliminary laboratory work is necessary.

New treatment technologies are urgently needed for AMD. Microbial mitigation of acid water using cellulosic materials is a potentially effective treatment. Now that we have a laboratory model that simulates field conditions, and a field test site where our hypotheses can be verified, the full potential of this new treatment can be determined, and an effective treatment process can be developed.

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Figure 1

Microbial Neutralization of AMD Circulating Through Troughs Containing Cellulosic Materials in the Laboratory

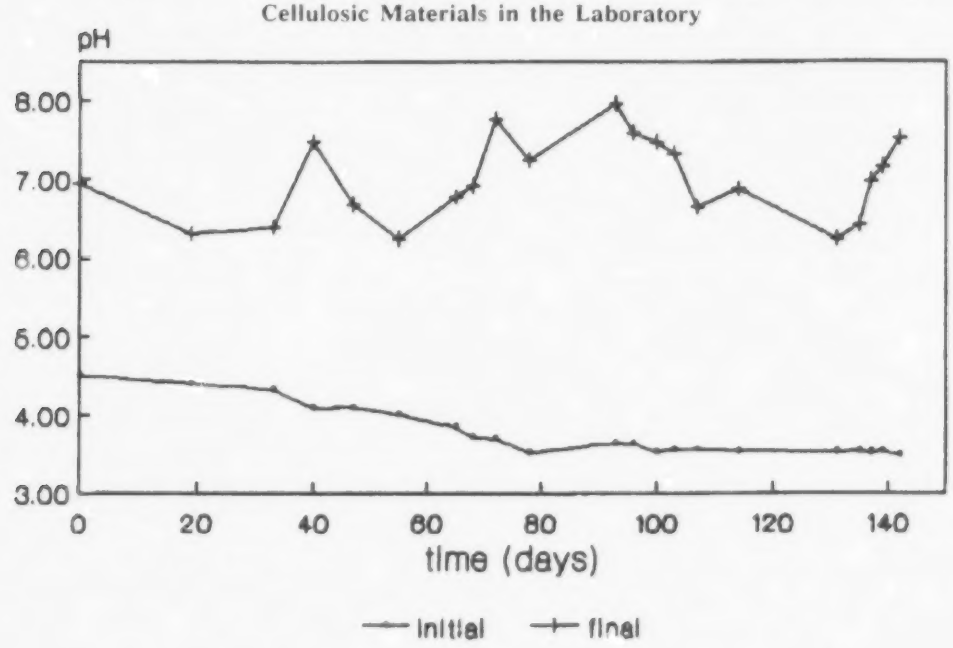


Figure 2

Removal of Sulphate From AMD After Passage Through Troughs in the Laboratory

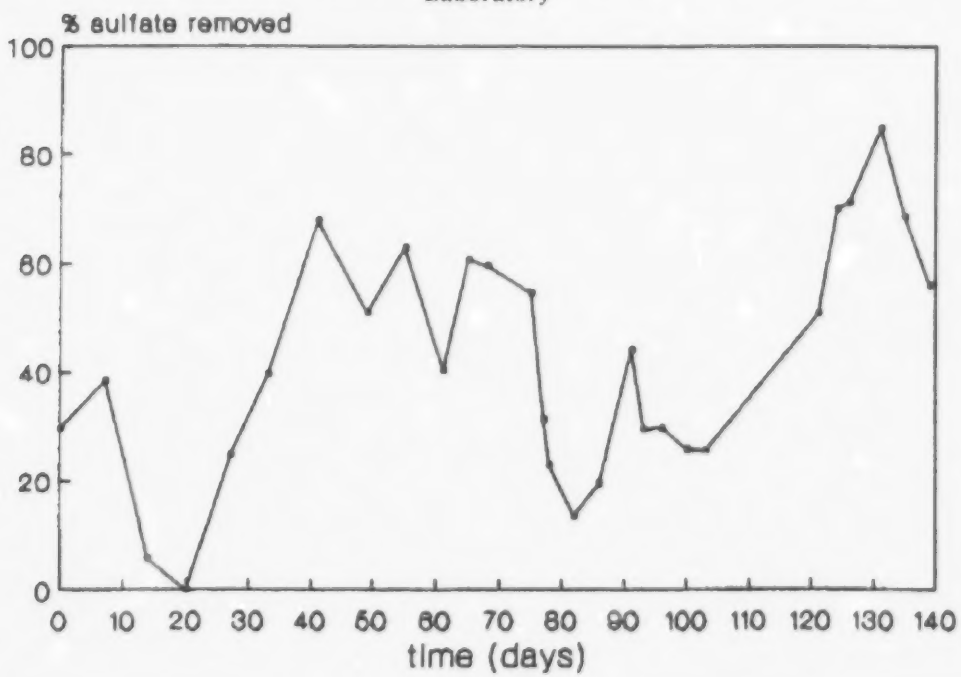


Figure 3

First Field Test at Halifax Airport: pH Values Measured in the Fall 1988

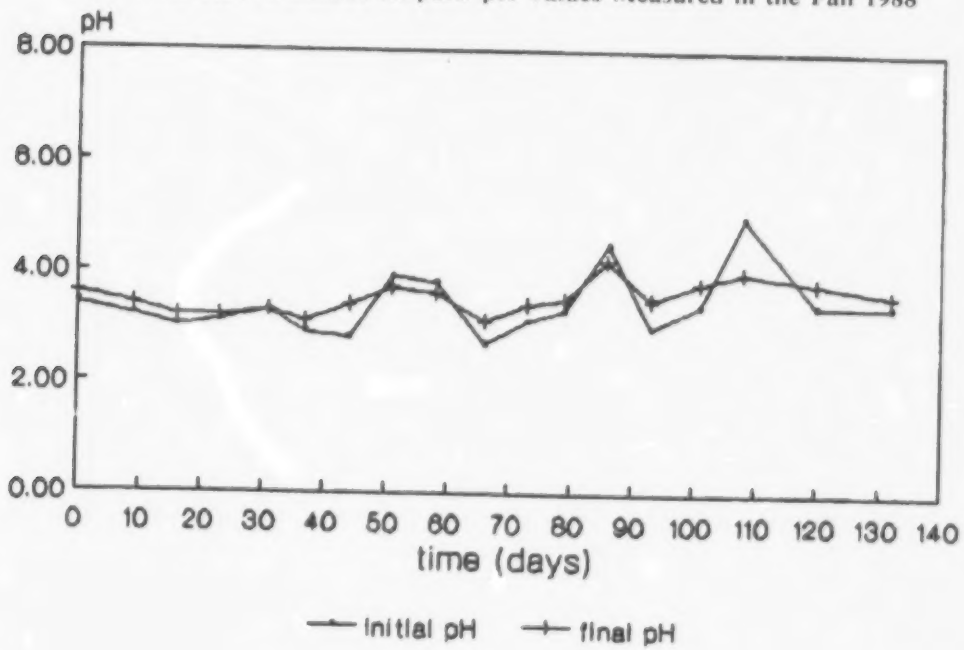


Figure 4

Microbial Counts for Samples Taken From Site A1 in the First Cell in 1988

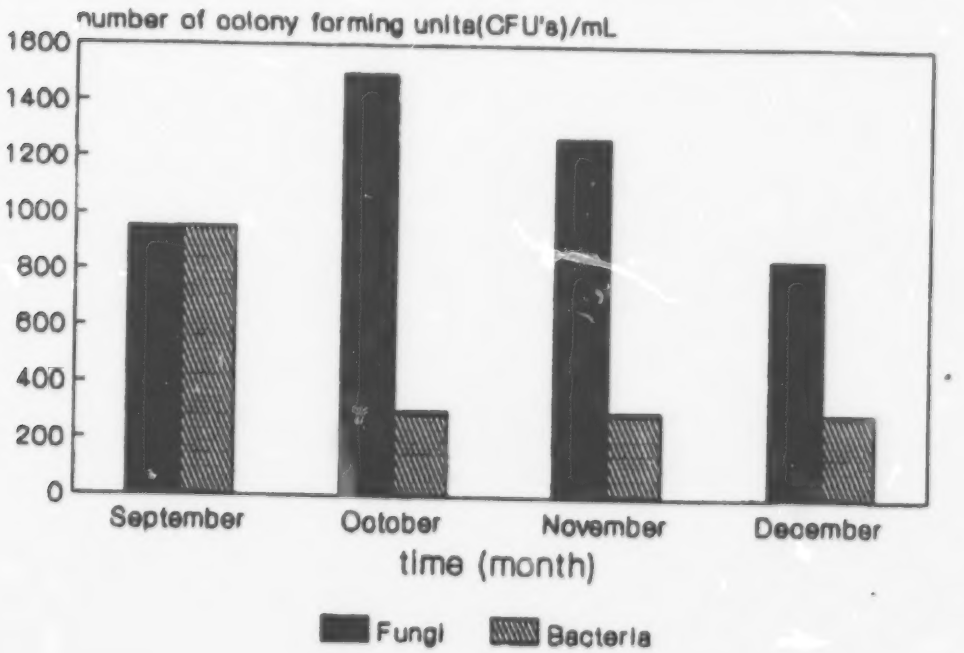


Figure 5

Second Field Test at Halifax Airport: pH Values Measured in the Fall 1989

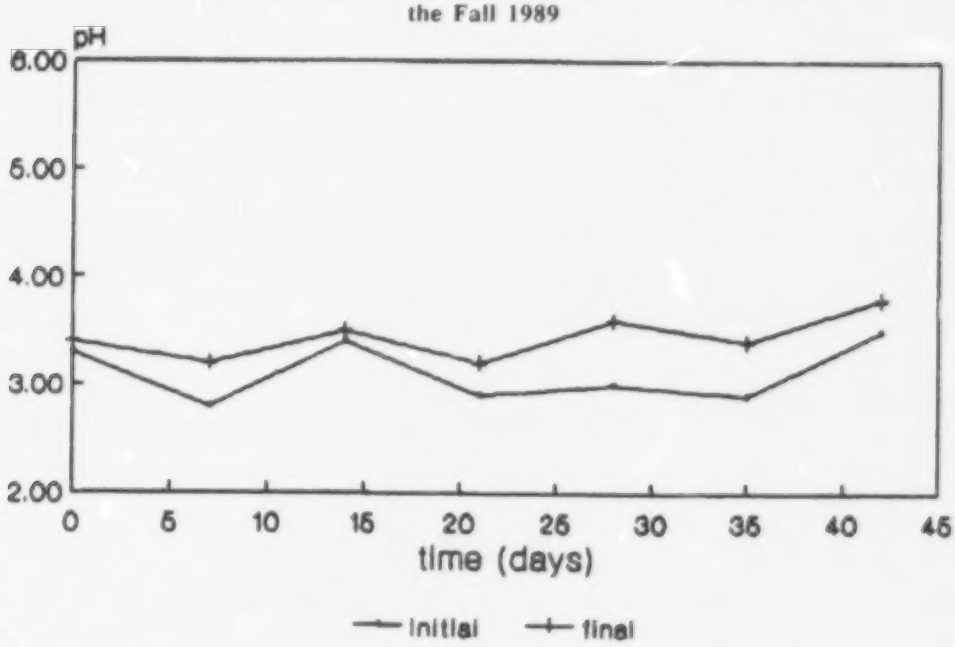
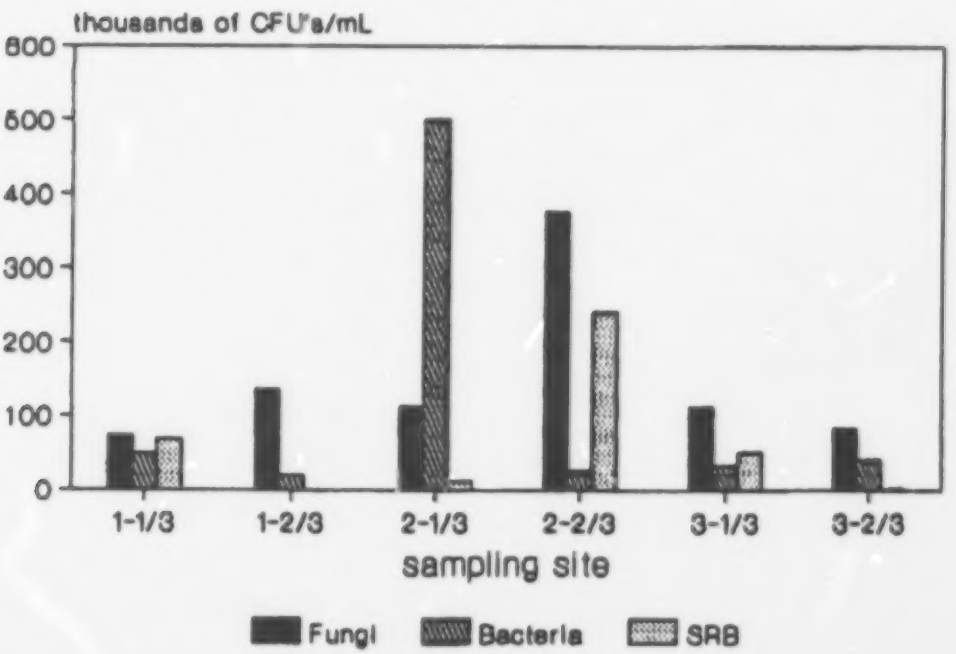


Figure 6

Microbial Counts for Samples Taken from One Month After Startup of Second Field Test



Further Studies on the Distribution and Fate of Arsenic and Mercury at a Site Contaminated by Abandoned Gold Mine Tailings

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Abstract

Gold mining has been conducted in Nova Scotia since the mid-1800s. Tailings from most of these operations were simply dumped near the sites, often in stream beds. Arsenic found in arsenopyrite in crushing-mill feed, along with elemental mercury lost during the gold extraction process (amalgamation), eventually entered surface- and groundwaters and affected surrounding vegetation and aquatic communities. Studies presented by Lane *et al.* at the 1988 BIOMINET meeting suggested that distributions of aquatic and semi-aquatic plant communities were not related to toxic metal levels. However, insufficient sampling of the physical-chemical environment at the tailings site at Black Brook (near Oldham, Nova Scotia) negated any clear definition of the influence of high metal concentrations on plant distribution. To ascertain whether the plants were tolerant of high metal levels, or if differences in the availability of metals around the site had any bearing on plant distribution, additional chemical and mineralogical measurements were performed at all 123 sampling sites.

Weathering of surface tailings by various physical, chemical and biological processes yields a supply of fine particles enriched in mercury and arsenic. Surface runoff and groundwater flow mobilizes arsenic (and manganese and iron) but apparently leaves most of the mercury behind. Wind and fluvial transport of mercury associated with a soil-float fraction (clays and fine organic particles) led to high soil concentrations near water courses and in the downwind side of vegetation barriers. Highest concentrations were recorded in areas with the most vegetation and highest soil organic matter and water contents.

Cluster analysis of the vegetation data suggested that there are 10 distinct communities on the tailings. Discriminant analysis of the physical and chemical characteristics of the tailings revealed that these plant communities were probably differentiated based on how deep it was to the water table and the texture of the tailings as measured by organic matter content and soil bulk density. Over the relatively small area examined, neither total nor leachable arsenic and mercury concentrations appeared to have any influence on the distribution of plant species. About 80% of the plant species present in sufficient abundance to be included in the discriminant analysis appeared to be insensitive to the range of arsenic and mercury concentrations found in the tailings area (70 to 26,000 ppm total arsenic, and 0.7 to 8.9 ppm total mercury). That some species found on the tailings can tolerate and even accumulate metals suggests aquatic and semi-aquatic plants may be useful for reclamation and treatment of mine tailings.

Résumé

En Nouvelle-Écosse, on extrait de l'or depuis le milieu du 18e siècle. Les résidus de la plupart des exploitations étaient simplement empilés près des mines, et souvent déversés dans les cours d'eau. L'arsénic contenu dans l'arsénopyrite alimentant les broyeurs, ainsi que le mercure élémentaire perdu durant l'extraction de l'or (amalgamation), finissaient par aboutir dans les eaux superficielles et souterraines et affecter ainsi la végétation et les communautés aquatiques environnantes. Selon les études présentées par Lane *et al.* à la réunion de BIOMINET, la distribution des communautés aquatiques et semi-aquatiques n'est pas liée aux concentrations de métaux toxiques. Toutefois, l'échantillonnage du milieu physico-chimique du site d'entreposage des résidus à Black Brook (près d'Oldham en Nouvelle-Écosse) a été trop restreint pour définir nettement l'influence des fortes concentrations de métaux sur la distribution des plantes. Pour s'assurer que les plantes peuvent tolérer de fortes concentrations de métaux, ou que les différences de concentration autour du site influent sur la distribution des plantes, on a effectué des mesures chimiques et minéralogiques supplémentaires aux 123 sites d'échantillonnage.

L'altération des résidus de surface par divers processus physiques, chimiques et biologiques produit une quantité de particules fines enrichies de mercure et d'arsénic. Les eaux de ruissellement et l'eau souterraine mobilisent l'arsénic (et le manganèse et le fer) mais pas, semble-t-il, le mercure. Le transport éolien et fluvial du mercure, associé à une fraction de flottation du sol (argiles et particules organiques fines), a produit des concentrations élevées du sol près des cours d'eau et en aval des écrans de végétation. Les plus fortes concentrations ont été enregistrées dans les zones où la végétation est la plus abondante et les teneurs en matières organiques et en eau du sol sont les plus élevées.

Une analyse typologique des données sur la végétation a révélé la présence de 10 communautés différentes sur les résidus. Une analyse discriminante des caractéristiques physiques et chimiques des résidus a révélé que ces communautés de plantes différaient probablement selon leur profondeur par rapport à la nappe phréatique et selon la texture des résidus déterminée par la teneur en matières organiques et la densité apparente du sol. Dans la zone analysée qui était relativement petite, aucune concentration d'arsénic et de mercure total ou lixiviable ne semble avoir influé sur la distribution des espèces de plantes. Environ 80% des espèces de plantes qui étaient suffisamment abondantes pour être incluses dans l'analyse discriminante n'étaient pas affectées par les diverses concentrations d'arsénic et de mercure contenues dans la zone de résidus (de 70 à 26 000 ppm d'arsénic total et entre 0,7 et 8,9 de mercure total). Le fait que certaines espèces poussant sur les résidus puissent tolérer et même accumuler des métaux, laisse supposer que les plantes aquatiques et semi-aquatiques pourraient être utiles pour restaurer et traiter les résidus miniers.

Introduction

Surface- and groundwater that flow through and over waste tailings from mining operations mobilize various metals for downstream transport and biological uptake. Runoff from many of these sites are often very acidic because of oxidation of pyrite associated with the metal deposits. This in turn allows further metal solubilization and adds more stress to indigenous plant and animal communities. Many plants, especially aquatic and emergent species, were seen growing and apparently flourishing near tailings deposits. This led to the construction of numerous "artificial wetlands" to treat acid mine drainage (AMD). Unfortunately very little was known about the physiology and ecology of the indigenous plant communities, and as a consequence, most of the engineered projects did not work as expected (Kleinmann, 1987).

To examine the response of plant communities to metal-contaminated soils and tailings, P. Lane and Associates, under contract to Energy, Mines and Resources Canada, conducted vegetation surveys and physical-chemical characterization of the soils, tailings and water (surface- and groundwater) at an abandoned gold mine in Nova Scotia. As was typical of gold mining operations in the late 1800s and early 1900s, most of the waste tailings were dumped at the mill sites, often directly into stream beds. Soils and vegetation that eventually developed over the tailings contain very high arsenic and mercury concentrations. Arsenic comes from arsenopyrite in gold-bearing quartz veins, while mercury levels result from losses incurred during amalgamation of gold.

At last year's BIOMINET meeting, Lane *et al.* (1988) reported no apparent effect of high concentrations of arsenic and mercury on plant distribution patterns. Plants growing there were either very tolerant of the metals, or able to exclude them from their tissues. The statistical analyses used, however, incorporated only physical-chemical data from 37 of the total 123 sampling locations. Chemical characterization of the soils and tailings was also limited to analyses of total metal concentration resulting from strong acid digestions. Considering that plants have access to only the water soluble fraction (which mainly comes from fine particulate matter), further studies were needed to test the conclusions of Lane *et al.* (1988) and to determine which plant species could be used for experimental studies and possible field applications. This paper presents the results of additional chemical and statistical analyses performed on soil and vegetation samples collected during the 1988 study, and shows how plant communities may be useful for treating AMD.

Methods and Materials

The Study Site: The Oldham site in Nova Scotia is 30 km northeast of Halifax, in the upper reaches of the Shubenacadie River watershed. High-grade ore deposits at this site led to practically continuous gold mining operations from 1862 to 1910 and 1936 to 1942. The resulting layer of waste tailings, 0.7 to 1.5 m thick, was spread over about 5 ha. Two large bare spots at the site were a result of extensive recreational vehicle traffic (not high metal concentrations). A small tributary of the Shubenacadie River, Black Brook, runs through the middle of the deposits and is fed by a small stream entering from the northeast corner of the deposits (see Figure 1). Black Brook also receives metal-rich, acidic waters from Halifax International Airport, just 3 km upstream from the site. AMD resulted from construction activities at the aiport that exposed massive beds of pyritic slates.

Soil and Vegetation Sampling and Analyses: At each of the 123 sampling locations shown in Figure 1, soil and vegetation samples were taken, and the depth to water table and thickness of the organic matter layer were determined. Samples of the upper 10 cm of soil were collected with a 6 cm diameter corer. At 37 sites, holes were dug into the tailings-layer and additional soil samples taken at distinctive layers. All herbaceous vegetation and shrubs and trees shorter than 50 cm were harvested from a 50 by 50 cm quadrat. Biomass of larger shrubs and trees were estimated within a 2 by 2 m quadrat by empirically derived regression equations that relate plant biomass to its diameter, 25 cm above ground (Freedman *et al.*, 1982; Crowell, 1988; Lane *et al.*, 1988).

In the laboratory, vegetation samples were sorted by species, dried and weighed to yield species-specific biomass. Tissue concentrations of arsenic and mercury were measured only in the most important species as determined by discriminant analysis of the complete data set (see below). Soil samples were dried and weighed to provide measures of soil bulk density. Subsamples were then taken for determining the percentage of organic matter content (weight loss on ignition at 550°C), and total and water-soluble (leachable) arsenic and mercury concentrations. Other subsamples were sieved through a #4 screen (4.75 mm) to remove coarse organic matter and then further separated into "soil-float" and "soil-sink" fractions by a series of distilled water rinses. This latter process separated particles with a specific density of more than 1.0 (sand and silt) from fine clays and organic matter. Both fractions were dried overnight at 80°C, weighed and analyzed for total and leachable arsenic and mercury. Mineralogical and metal analyses were also carried out on five size fractions separated from samples collected at four of the soil profile stations. Samples were separated by standard sedimentological sieves into a clay-plus-silt fraction and four phi-units in the sand-sized range.

Arsenic and mercury concentrations in soil and plant samples were performed by standard techniques (colourimetric and atomic absorption methods) at the Mineral Engineering Centre of the Technical University of Nova Scotia. Total metal concentrations in soil samples resulted from digestion of 0.5 g samples by aqua-regia (arsenic) or concentrated nitric and hydrochloric acids (mercury). To determine leachable values, 1 g samples were added to 50 mL of distilled water and stirred for 0.5 h. Filtrates of these mixtures were then subjected to acid digestions. For determining metal concentrations in plant tissues, the same methods outlined above were used, except concentrated nitric acid was used for digesting the tissues.

Water Chemistry: Surface water was collected at five locations in and around the tailings site during June, August and November 1988 (see Figure 1). Six piezometers were buried in the tailings (see Figure 1) to provide access to groundwaters. All water samples were subsequently analyzed for a large suite of metals, major ions and other limnological parameters at the Environmental Chemistry Laboratory of the Victoria General Hospital in Halifax. As much of this data has already been presented (Lane *et al.*, 1988), only the highlights of the arsenic, mercury, manganese, iron, aluminum, calcium and pH data will be presented.

Statistical Analyses: All statistical analyses were done using SPSS/PC+ (version 2.0). The vegetation data set was initially transformed (ln species-specific biomass + 1) and separated into sub-groups by the CLUSTER program (squared Euclidean distance and centroid method of clustering specified). Discriminant analysis of the vegetation data and the combined physical-chemical and vegetation data sets

were then performed to examine the consistency of cluster assignments and identify those variables that appear to be responsible for the statistical groupings.

Results and Discussion

Physical–Chemical Environment: Water samples upstream from the tailings, midway through, and downstream from the deposits in Black Brook (a total distance of about 1 km), clearly show a scavenging of arsenic and manganese on the two or three sampling periods (see Figure 2). Some iron may also be stripped from this site according to results from the August sampling period (see Figure 3) and from comparisons between the small feeder stream entering the northeast part of the tailings and the pond samples (see Figure 1 for locations). High manganese, iron and aluminum values (about 0.5 ppm), and a low pH (see Figure 3) at the upstream station, reflect AMD from the exposed slate beds at Halifax Airport. By contrast, metal concentrations in the small feeder stream were generally at or below limits of detection (<0.03 ppm arsenic, <0.01 ppm manganese, <0.1 ppm iron and <0.05 ppm aluminum), and pH values were around 7.3. As the water flows through the tailings, there is an apparent amelioration of water acidity (see Figure 3). Solubilization of calcite and feldspars in the waste tailings increased both pH and calcium concentrations. This was particularly noticeable on the June survey, when pH rose from 4.3 to 6.3 over the 1 km stretch. Mercury was consistently below the limit of detection (<0.05 ppm) for all stations and sampling periods.

Groundwater concentrations of arsenic, manganese and iron were often an order of magnitude higher than surface water values (see Table 1). Variability in metal concentrations seemed to be primarily linked to positioning of the piezometers around the site. Two of the piezometers had low but similar results, apparently because of placement near the edge of the tailings or the bank of Black Brook. Mercury was below limits of detection, suggesting little solubilization and downstream transport.

A typical soil profile at the Oldham site usually contained three distinct layers (see Figure 4). An organic–rich, fine–grained soil, 5 to 15 cm in thickness, covered much of the tailings (exceptions were related to heavy recreational vehicle traffic). The tailings themselves were primarily coarse sands with a scattering of rock fragments. The thickness of tailings varied considerably around the site, but averaged about 75 cm. In some locations, a double layer of tailings, separated by an organic soil, was observed. The soil accumulated in the 25–year gap between the two main phases of mining activity. A gray clay layer was usually found beneath the tailings.

Chemical analyses of soils and tailings (see Figure 4, Table 2), together with the water chemistry data, suggest that arsenic, and probably manganese, iron and aluminum, can be removed from the site by two main processes: solubilization, and wind and fluvial transport of metal–rich particles. Redistribution of mercury seems to occur only by mass transport of fine particles. Both arsenic and mercury were enriched in the soil–float fraction of a sample, that is, in clays and fine organic particles that were suspended in distilled water rinses of samples (see Table 2). Metal concentrations in the fines were three to six times higher than in heavier sands and silts that made up the soil–sink fraction. As a consequence, variations in soil metal concentrations around the site appeared to be a function of dominant grain–size of the soils, extent of vegetation cover and topographic relief. Highest mercury values were generally found near water courses (streams or ponds) and downwind of thick stands of vegetation. Arsenic distributions did not follow this pattern because of faster rates of solubilization of fine particles, especially in wet areas.

Biologically mediated removal of arsenic and mercury undoubtedly occurred. Microbial methylation reactions, in particular, could be an important pathway (Rogers, 1975; Cox, 1975). Arsenates and elemental mercury are reduced by bacteria and fungi to yield volatile methyl–arsines and methyl–mercury, which subsequently blows off to the atmosphere. High concentrations of volatile organo–metals in the air around sewage treatment plants (Myers *et al.*, 1973; Soldano *et al.*, 1975) suggests that further work on optimizing methylation processes might be possible with bio–engineered systems for AMD that contains high levels of arsenic and mercury.

Plant Communities Living on the Tailings: As noted by Lane *et al.* (1988), most of the tailings were covered with vegetation. Species at this site were common throughout Nova Scotia. Cluster analysis of the vegetation data set indicated there were 10 distinct subgroupings of plants. Table 3 is a list of 33 species common in at least 1 of the 10 communities found on the tailings. The criterion for inclusion in the list was a frequency of occurrence of at least 30% in at least one of the plant communities. This eliminated species whose presence may have been the result of chance rather than that species' ability to survive under the environmental conditions prevalent there.

Cluster 1 represents a shallow pond community dominated by *Chara globularis*, the horsetail *Equisetum fluviatile* and *Utricularia intermedia*. The presence of permanent water appears to be the main determinant of the species composition.

Cluster 2 was dominated by the rush *Juncus articulatus* and *E. fluviatile*. These species accounted for 84% of the total above–ground biomass. Most of the study sites had water tables close to the surface, which encouraged organic matter to accumulate.

Cluster 3 was dominated by the moss *Campylium stellata* and *E. fluviatile* (83% of the total biomass).

Cluster 4 was dominated by the horsetails *E. arvense* and *E. fluviatile*, and the grass *Agrostis palustris*. These three species accounted for 74% of the total biomass of the community.

Clusters 5 and 6 were less distinct than most of the other communities and contained different mixtures of species dominating Clusters 3 and 4 (*E. fluviatile*, *E. arvense*, *A. palustris* and *C. stellata*).

Cluster 8 was heavily dominated by the alder *Alnus rugosa*, *Spiraea latifolia* and *Aster umbellatus* (97% of total biomass). This community was characterized by a well–developed overstory of alders and hence was named the tall alder community.

Cluster 9 was named the short alder community and appeared to be an earlier successional stage of cluster 8. Species' composition and dominants were nearly identical, only the heights and biomass seemed to differ.

Cluster 10 was dominated by relatively stunted alders, *S. latifolia* and *E. fluviate* (86% of the biomass). This community was restricted to swampy areas, and was called the wet alder community.

Cluster 11 was another poorly defined cluster. Dominant species included alders, the red maple *Acer rubrum*, *S. latifolia*, the fern *Onoclea sensibilis* and *E. arvense*.

The most abundant species were apparently very tolerant to arsenic and mercury and grew throughout the entire range of metal concentrations. The most widely spread plants on the tailings–horsetails, the

grass *A. palustris*, mosses and rushes–are known to be tolerant of high arsenic concentrations or other heavy metals (Porter and Peterson, 1977; Brooks *et al.*, 1981; Dale and Freedman, 1982; Dollhopf *et al.*, 1988). Plots of their biomass against total arsenic and mercury concentrations in the soils they were living in all indicate no apparent limitation (see figures 5 to 8). Similar plots with leachable metal concentrations did not provide any new information (compare figures 8 and 9). As noted in other studies (National Research Council of Canada, 1978; Sherbin, 1979), there were no obvious relationships between total and leachable concentrations because of differences in soil constituents able to bind metals (i.e., clays, organic matter and various metal oxides; see Figure 10). Artificial leaching of soil samples with distilled water released, on average, 7% of the total arsenic but only 2% of the total mercury (see Table 2).

Discriminant analysis of the physical–chemical and vegetation data sets revealed that the 10 plant communities were probably differentiated on the basis of depth to water table, soil organic matter content and soil bulk density (see Figure 11). These three physical variables explained 69% of the variance in the vegetation data set. Adding soil metal concentrations to the analysis increased the per cent variance explained by only 10%, perhaps of questionable significance. Canonical correlations suggested that clusters 3 to 6, and clusters 8, 9 and 11 may be differentiated by soil metal concentrations.

Only 7 of the 33 species found in sufficient abundance to be included in the discriminant analysis seemed to be limited by soil metal concentrations (*Carex brunessens*, *Iris versicolor*, *Juncus effusus*, *Rubus pubescens*, *Solidago canadensis*, *Solidago rugosa* and *Thalictrum polygamum*: see tables 4 and 5). These species, all comparatively minor components for biomass, were observed only at locations with less than approximately 100 ppm leachable arsenic (out of a range of 1 to 750 ppm) and less than 0.1 ppm leachable mercury (total range of <0.01 to 0.3 ppm). Plant tissue concentrations were also quite low in these species, indicating some ability to exclude the metals (see Table 6).

The other 26 species were very tolerant to soil metals and most readily took up the metals into their tissues (see Table 6). Mosses (*Campylium* and *Polytrichum*) and other aquatic plants such as *Chara*, filamentous green alga *Microspora*, and the bur reed *Sparganium* (see Table 7) were particularly adept at accumulating arsenic and mercury. Arsenic concentrations in horsetails and *Microspora* collected at the tailings site were two orders of magnitude higher than values normally observed in plants living in uncontaminated waters (Dale and Freedman, 1982). Many of the metal loadings, however, may be attributed to adsorption onto the plant surfaces (or uptake by epiphytic bacteria) rather than incorporation into plant tissues. Preliminary studies of the partitioning of arsenic and mercury within plants indicate substantial metal adsorption onto surfaces that are constantly exposed to metal–enriched waters (see Table 7). The roots of five different plant species had 4 to 17 times higher metal concentrations as compared with above–ground shoots.

Conclusions

Statistical associations within the large body of physical, chemical and vegetation data indicate the extreme tolerance of plant communities living on gold mine tailings to high concentrations of arsenic and mercury, and their ability to adapt to poor situations. We still do not know how these tailings influence plant succession or the time required for adaptations to metal–rich soils. Our ongoing laboratory experiments should give more information about tolerances and adaptability of plant species that are typical of the

Maritimes. It is not known whether these species will be useful in other regions, or with different types of mine wastes. Studies by Kalin (e.g., Kalin and Smith, 1987; Kalin and Scribailo, 1988) suggest that *Chara* and *Typha* may be useful at some Ontario sites (especially those with alkaline effluents), but these species are either uncommon or not as hardy at the Oldham site in Nova Scotia. We also need to know whether plants (or seed) from uncontaminated areas can be successfully transplanted to tailings sites. Clearly, much more work is needed on the ecology and physiology of plant species growing on or near mine tailings.

The best candidates for treating acid and alkaline mine drainage must be able to incorporate or adsorb all types of metals, grow quickly and remain at the site. Our results indicate that plant communities:

- reduce erosive loss of contaminated soils and tailings;
- mechanically trap fine, metal-rich particles; and
- contribute labile organic matter to soils, which initiates further plant succession and stimulates microbial methylation of arsenic and mercury and the production of metal-binding sulphides. Removing plant cover to recover metals or remove potentially toxic foods from wildlife browsers could rapidly alter downstream water quality.

Acknowledgements

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Table 1

Heavy Metal Concentrations in Ground Water from Oldham Tailings Site

	Metal Concentrations (ppm) at Six Piezometer Stations		
	P_1 P_3 P_4 P_5	P_2^* P_6^*	Concentration compared to surface water
Arsenic	14±8	2±1	ca. 100x
Mercury	<0.05	<0.05	equal
Manganese	4±2	2	ca. 20x
Iron	38±23	3±1	up to 10x

* Tailings-edge and river-edge stations

Table 2

Total and leachable concentrations (ppm) of Arsenic and Mercury in 123 surface soil samples. Measurements were made on unprocessed soil samples and on the soil-float and soil-sink fractions. Values in brackets are standard deviations.

		Unprocessed	Soil-Float	Soil-Sink
Arsenic	Total	2000	4900	1000
		(3500)	(8000)	(2000)
	Leachable	60	110	40
		(110)	(180)	(80)
Mercury	Total	3.4	6.4	2.2
		(1.9)	(3.0)	(1.6)
	Leachable	0.05	0.11	0.02
		(0.05)	0.12	0.02

Table 3

Mean species biomass and frequency of occurrence for all common species in each of 10 plant communities. Plant community codes are as follows: C.g.–E.f.=Chara globularis – Equisetum fluviatile, J.a.–E.f.=Juncus articulatus – E. fluviatile, C.s.–Ef.=Campylium stellata – E. fluviatile, E.a.–A.p.=Equisetum arvense – Agrostis palustris, E.f.–A.p.=E. fluviatile – A. palustris, E.a.–E.f. = E. arvense – E. fluviatile, Tall A.r. S.I. = Tall Alnus rugosa – Spiraea latifolia, Short A.r.–S.I.=Short A. rugosa – S. latifolia, Wet A.r.–S.i.=Wet A. rugosa – S. latifolia an A.r.–A.r.=A. rugosa – Acer rubrum

	Cluster 1 C.g-E.f.		Cluster 2 J.a-E.f		Cluster 3 C.s.-E.f.		Cluster 4 E.f.-A.p.	
Species	Biomass g/m^2	Frequency (%)	Biomass g/m^2	Frequency (%)	Biomass g/m^2	Frequency (%)	Biomass g/m^2	Frequency (%)
Acer rubrum	-	-	1	10	-	-	<1	10
Agrostis paluatris	-	-	1	57	16	100	31	100
Alnus rugosa	-	-	1	10	-	-	-	-
Aster novi-belgii	-	-	1	33	1	71	10	70
Aster umbellatus	-	-	-	-	-	-	-	-
Campylium stellata	-	-	-	-	200	95	3	30
Carex brunescens	-	-	1	10	-	-	-	-
Carex viridula	-	-	1	33	2	57	-	-
Centaurea nigra	-	-	<1	5	4	43	<1	30
Chara globularis	100	100	1	10	-	-	-	-
Chrysanthemum leucanthemum	-	-	<1	5	1	38	1	20
Bryopteris thelypteris	-	-	1	14	3	14	2	10
Equisetum arvense	-	-	1	10	<1	10	33	100
Equisetum fluviatile	14	67	35	100	49	100	46	70
Equisetum variegatum	-	-	<1	5	7	57	3	50
Equisetum perfoliatum	-	-	1	20	1	52	8	80
Hypericum virginicum	-	-	1	57	<1	19	1	30
Juncus articulatus	-	-	110	90	3	48	2	60
Juncus effusus	-	-	-	-	-	-	-	-
Larix laricina	-	-	<1	5	<1	14	<1	20
Leontodon autumnalis	-	-	<1	5	<1	5	-	-
Lycopus uniflorus	-	-	1	48	1	67	1	30
Lysimachia terrestris	-	-	2	62	1	29	1	50
Onoclea sensibilis	-	-	<1	5	1	19	-	-
Picea glauca	-	-	-	-	-	-	-	-
Plantago major	-	-	-	-	<1	5	<1	10
Polytrichum commune	-	-	-	-	-	-	-	-
Prunella vulgaris	-	-	<1	19	3	57	<1	50
Rubus pubescens	-	-	-	-	-	-	-	-
Scirpus rubrotinctus	-	-	-	-	-	-	-	-
Spiraea latifolia	-	-	1	14	1	19	2	60
Utricularia intermedia	9	100	<1	10	-	-	-	-
Viola cucullata	-	-	<1	10	-	-	1	30
Viola pallens	-	-	<1	19	<1	38	1	80
Mean Total Biomass	120		170		300		150	

Table 3. Continued.

Species	Cluster 5 E.f.-A.p. Biomass g/m^2	Cluster 5 E.f.-A.p. Frequency (%)	Cluster 6 E.a.-E.f. Biomass g/m^2	Cluster 6 E.a.-E.f. Frequency (%)	Cluster 8 A.r.-S.t. Tall Biomass g/m^2	Cluster 8 A.r.-S.t. Tall Frequency (%)
Acer rubrum	2	29	-	-	1	29
Agrostis palustris	15	100	20	91	<1	12
Alnus rugosa	2	14	-	-	4800	100
Aster novi-belgii	7	71	3	64	<1	6
Aster umbellatus	-	-	-	-	8	71
Campylium stellata	13	86	36	45	<1	6
Carex brunescens	-	-	-	-	-	-
Carex viridula	<1	29	1	9	6	24
Centaurea nigra	2	57	<1	9	<1	12
Chara globularis	-	-	-	-	-	-
Chrysanthemum leucanthemum	4	71	1	36	-	-
Dryopteris thelypteris	<1	14	20	36	<1	6
Equisetum arvense	23	57	40	91	7	71
Equisetum fluviatile	50	100	38	100	1	59
Equisetum variegatum	10	71	6	64	<1	12
Eupatorium perfoliatum	16	57	21	73	-	-
Hypericum virginicum	-	-	2	18	<1	12
Juncus articulatus	19	43	7	55	<1	6
Juncus effusus	-	-	-	-	<1	6
Larix laricina	<1	29	<1	36	-	-
Leontodon autumnalis	-	-	1	36	-	-
Lycopus uniflorus	3	86	3	64	1	41
Lysimachia terrestris	2	14	1	55	<1	24
Onoclea sensibilis	2	14	-	-	4	47
Picea glauca	-	-	2	18	5	12
Plantago major	4	57	1	9	-	-
Polytrichum commune	-	-	19	27	-	-
Prunella vulgaris	5	86	3	27	<1	6
Rubus pubescens	-	-	1	9	4	53
Scirpus rubrotinctus	-	-	-	-	-	-
Spiraea latifolia	3	57	3	36	110	94
Utricularia intermedia	-	-	-	-	-	-
Viola cucullata	1	29	1	45	<1	18
Viola pallens	1	71	1	55	<1	29
Mean Total Biomass	190		260		5000	

Table 3. Continued.

Species	Cluster 9 A.r.-S.l. Short Biomass g/m^2	Frequency (%)	Cluster 10 A.r.-S.l. Wet Biomass g/m$_2$	Frequency (%)	Cluster 11 A.r.-A.r. Biomass g/m^2	Frequency (%)
Acer rubrum	3	10	9	40	360	67
Agrostis palustris	<1	30	-	-	10	67
Alnus rugosa	520	100	1200	100	2600	100
Aster novi-belgii	4	40	10	20	13	17
Aster umbellatus	35	70	7	40	9	67
Campylium stellate	16	50	10	20	20	50
Carex brunnescens	11	20	6	60	-	-
Carex viridula	1	20	<1	20	-	-
Centaurea nigra	-	-	-	-	<1	17
Chara globularis	-	-	-	-	-	-
Chrysanthemum leucanthemum	-	-	-	-	1	17
Dryopteris thelypteris	<1	10	-	-	-	-
Equisetum arvense	7	60	-	-	24	67
Equisetum fluviatile	9	70	9	100	6	67
Equisetum variegatum	-	-	-	-	<1	17
Eupatorium perfoliatum	2	30	-	-	6	50
Hypericum virginicum	1	60	10	60	1	17
Juncus articulatus	13	20	5	20	2	17
Juncus effusus	18	40	3	40	<1	17
Larix laricina	-	-	-	-	-	-
Leontodon autumnalis	-	-	-	-	<1	17
Lycopus uniflorus	<1	30	1	40	2	33
Lysimachia terrestris	4	60	1	40	-	-
Onoclea sensibilis	-	-	6	40	29	67
Picea glauca	-	-	-	-	330	33
Plantago major	-	-	-	-	<1	17
Polytrichum commune	34	30	10	40	1	33
Prunella vulgaris	-	-	-	-	-	-
Rubus pubescens	<1	10	<1	20	2	33
Scirpus rubrotinctus	1	10	-	-	9	50
Spiraea latifolia	120	90	180	80	120	67
Utricularia intermedia	-	-	-	-	-	-
Viola cucullata	<1	40	<1	20	1	67
Viola pallens	<1	70	<1	60	1	67
Mean Total Biomass	820		1600		3800	

Table 4. Soil arsenic concentrations for sites at which each of 33 species were found. SD = standard deviation.

Species	n	Total Arsenic (ppm) Mean (SD)	Range	Leachable Arsenic (ppm) Mean (SD)	Range
Maximum range			70 - 26000		1 - 750
Agrostis palustris	72	2100 (4000)	90 - 26000	70 (130)	1 - 750
Alnus rugosa	42	1500 (2000)	100 - 7600	40 (70)	1 - 420
Aster lateriflorus	13	1300 (1300)	90 - 4700	60 (40)	6 - 130
Aster novi-belgii	53	2100 (4500)	200 - 26000	80 (150)	1 - 750
Aster umbellatus	29	1800 (2200)	100 - 8600	50 (80)	2 - 420
Campylium stellata	50	1800 (2900)	90 - 1700	60 (120)	1 - 710
Carex brunessens	9	1900 (2900)	100 - 7600	20 (10)	2 - 40
Carex viridula	32	870 (1200)	130 - 6300	50 (130)	1 - 710
Centaurea nigra	22	1900 (5400)	90 - 26000	50 (70)	1 - 320
Chara globularis	8	460 (140)	310 - 700	20 (10)	5 - 40
Chrysanthemum leucanthemum	21	1400 (3600)	90 - 17000	40 (30)	2 - 120
Dryopteris thelypteris	16	3000 (6300)	200 - 26000	80 (90)	1 - 320
Equisetum arvense	53	2900 (4700)	90 - 26000	90 (130)	1 - 750
Equisetum fluviatile	105	2100 (3700)	70 - 26000	60 (110)	1 - 750
Equisetum variegatum	33	1700 (2800)	90 - 12000	90 (180)	1 - 750
Eupatorium perfoliatum	46	2700 (4900)	90 - 26000	80 (120)	1 - 750
Hypericum virginicum	38	3100 (4600)	100 - 26000	60 (80)	1 - 320
Iris versicolor	9	490 (370)	170 - 1300	20 (10)	6 - 40
Juncus articulatus	53	2100 (4000)	70 - 26000	50 (70)	1 - 320
Juncus effusus	11	3400 (3000)	480 - 8600	40 (40)	2 - 120
Lycopus uniflorus	64	1800 (4000)	70 - 26000	50 (70)	1 - 420
Lysimachia terrestris	47	2500 (4200)	70 - 26000	50 (70)	1 - 320
Onoclea sensibilis	21	2500 (3900)	90 - 17000	60 (90)	2 - 420
Osmunda regalis	8	2000 (2100)	350 - 3500	50 (70)	1 - 220
Polytrichum commune	12	3700 (3700)	290 - 12000	120 (210)	2 - 750
Prunella vulgaris	36	1100 (1600)	90 - 8600	70 (120)	1 - 710
Rubus pubescens	15	580 (400)	100 - 1700	30 (20)	7 - 110
Solidago canadensis	10	580 (450)	200 - 1700	30 (20)	6 - 60
Solidago rugosa	6	600 (260)	350 - 1000	20 (10)	7 - 40
Spiraea latifolia	55	1700 (2100)	90 - 8600	60 (70)	1 - 420
Thalictrum polygamum	23	1400 (3400)	100 - 1700	30 (20)	6 - 120
Viola pallens	55	2600 (4600)	90 - 26000	70 (110)	1 - 750

Table 5. Soil mercury concentrations for sites at which each of 33 species was found. SD = standard deviation.

Species	n	Total Mercury (ppm) mean (SD)	Total Mercury (ppm) range	Leachable Mercury (ppm) mean (SD)	Leachable Mercury (ppm) range
Maximum range			0.7 - 8.9		<0.01 - 0.29
Agrostis palustris	72	3.4 (1.9)	1.1 - 8.9	0.05 (0.05)	<0.01 - 0.29
Alnus rugosa	42	3.4 (2.0)	0.7 - 8.9	0.04 (0.05)	<0.01 - 0.29
Aster lateriflorus	13	3.1 (1.6)	1.2 - 6.9	0.08 (0.06)	0.02 - 0.21
Aster novi-belgii	53	3.3 (1.7)	1.0 - 8.4	0.05 (0.06)	<0.01 - 0.29
Aster umbellatus	29	3.7 (2.4)	1.0 - 8.9	0.06 (0.07)	<0.01 - 0.29
Campylium stellata	50	3.2 (1.8)	1.1 - 8.4	0.06 (0.06)	0.01 - 0.29
Carex brunessens	9	2.8 (1.7)	1.0 - 6.6	0.02 (0.02)	<0.01 - 0.04
Carex viridula	32	2.7 (1.3)	0.7 - 7.5	0.04 (0.03)	<0.01 - 0.11
Centaurea nigra	22	3.2 (1.5)	1.3 - 7.2	0.05 (0.05)	0.01 - 0.20
Chara globularis	8	4.5 (1.9)	2.6 - 8.4	0.03 (0.02)	0.01 - 0.06
Chrysanthemum leucantheum	21	2.7 (1.6)	1.3 - 8.2	0.05 (0.06)	<0.01 - 0.23
Dryopteris thelypteris	16	3.4 (1.5)	1.6 - 7.2	0.06 (0.05)	0.01 - 0.21
Equisetum arvense	53	3.8 (2.1)	1.1 - 8.9	0.06 (0.06)	<0.01 - 0.29
Equisetum fluviatile	105	3.4 (1.9)	0.7 - 8.9	0.05 (0.05)	<0.01 - 0.29
Equisetum variegatum	33	2.9 (1.8)	1.1 - 8.9	0.05 (0.05)	<0.01 - 0.23
Eupatorium perfoliatum	46	3.6 (2.1)	1.1 - 8.9	0.04 (0.05)	<0.01 - 0.23
Hypericum virginicum	38	4.0 (2.0)	1.1 - 8.9	0.03 (0.05)	<0.01 - 0.11
Iris versicolor	9	2.2 (0.9)	1.1 - 4.3	0.02 (0.02)	0.01 - 0.06
Juncus articulatus	53	3.3 (1.7)	1.1 - 8.9	0.03 (0.04)	<0.01 - 0.21
Juncus effusus	11	5.0 (2.1)	1.5 - 7.5	0.03 (0.02)	<0.01 - 0.08
Lycopus uniflorus	64	3.2 (1.9)	0.7 - 8.9	0.05 (0.05)	<0.01 - 0.29
Lysimachia terrestris	47	3.5 (1.9)	1.1 - 8.9	0.04 (0.05)	<0.01 - 0.29
Onoclea sensibilis	21	3.9 (2.5)	1.0 - 8.9	0.06 (0.06)	<0.01 - 0.27
Osmunda regalis	8	3.8 (2.4)	1.1 - 8.9	0.09 (0.10)	0.01 - 0.27
Polytrichum commune	12	4.8 (2.2)	1.0 - 8.4	0.09 (0.08)	<0.01 - 0.29
Prunella vulgaris	36	2.9 (1.2)	1.3 - 5.7	0.05 (0.05)	<0.01 - 0.21
Rubus pubescens	15	2.7 (1.6)	1.1 - 6.1	0.04 (0.02)	0.01 - 0.09
Solidago canadensis	10	2.2 (0.6)	1.5 - 3.4	0.04 (0.07)	<0.01 - 0.23
Solidago rugosa	6	3.2 (1.0)	2.1 - 4.7	0.04 (0.03)	<0.01 - 0.06
Spiraea latifolia	55	3.4 (1.8)	0.7 - 8.9	0.05 (0.06)	<0.01 - 0.29
Thalictrum polygamum	23	2.5 (1.6)	1.1 - 8.2	0.02 (0.02)	<0.01 - 0.06
Viola pallens	55	3.6 (2.0)	1.1 - 8.9	0.05 (0.06)	<0.01 - 0.29

Table 6. Tissue concentrations of arsenic and mercury in plant species found on the Oldham tailings. SD = standard deviation.

Species	n	Arsenic (ppm) Mean(SD)	Arsenic (ppm) range	Mercury (ppm) Mean(SD)	Mercury (ppm) range
Agrostis palustris	43	54 (86)	4-400	0.17 (0.12)	0.06-0.49
Alnus rugosa	30	35 (59)	4-300	0.13 (0.04)	0.07-0.27
Aster lateriflorus	8	9 (7)	3-20	0.07 (0.04)	0.02-0.11
Aster novi-belgii	26	6 (5)	3-20	0.16 (0.11)	0.04-0.42
Aster umbellatus	24	5 (3)	2-10	0.11 (0.08)	0.04-0.41
Campyllium stellata	36	220 (280)	3-1500	2.10 (1.76)	0.56-8.86
Carex brunessens	6	6 (4)	2-10	0.06 (0.03)	<0.01-0.07
Carex viridula	8	14 (12)	2-40	0.19 (0.14)	0.05-0.42
Centaurea nigra	13	7 (8)	2-30	0.19 (0.12)	0.08-0.50
Chara globularis	8	1700 (3400)	400-10,000	0.45 (0.11)	0.32-0.67
Chrysanthemem leucanthemum	11	44 (75)	2-200	0.18 (0.10)	0.03-0.33
Dryopteris thelypteris	12	14 (33)	3-100	0.13 (0.05)	0.07-0.24
Equisetum arvense	38	16 (25)	4-100	0.34 (0.26)	0.07-1.13
Equisteum fluviatile	74	45 (91)	3-500	0.23 (0.20)	0.03-1.47
Equisetum variegatum	22	27 (74)	2-300	0.11 (0.06)	0.04-0.25
Eupatorium perfoliatum	27	60 (89)	3-300	0.26 (0.30)	0.02-1.43
Hypericum virginicum	13	21 (31)	3-100	0.16 (0.15)	0.04-0.40
Iris versicolor	7	16 (29)	2-80	0.04 (0.02)	0.01-0.07
Juncus articulatus	31	16 (27)	3-100	0.10 (0.08)	0.01-0.42
Juncus effusus	9	5 (3)	3-10	0.06 (0.04)	0.03-0.13
Lycopus uniflorus	17	14 (20)	2-80	0.19 (0.12)	0.03-0.58
Lysimachia terrestris	21	10 (9)	3-40	0.14 (0.09)	0.04-0.45
Onoclea sensibilis	15	5 (8)	3-30	0.16 (0.09)	0.06-0.32
Osmunda regalis	5	4 (2)	3-10	0.08 (0.03)	0.04-0.10
Polytrichum commune	8	420 (800)	3-2400	2.26 (1.62)	0.43-4.49
Prunella vulgaris	13	16 (23)	2-80	0.26 (0.14)	0.14-0.64
Rubus pubescens	10	16 (41)	2-100	0.10 (0.05)	0.05-0.23
Solidago canadensis	7	3 (1)	2-4	0.06 (0.04)	0.02-0.14
Solidago rugosa	6	2 (1)	2-3	0.04 (0.02)	<0.01-0.05
Spiraea latifolia	34	26 (29)	2-100	0.16 (0.11)	0.07-0.67
Thalictrum polygamum	8	5 (3)	2-10	0.08 (0.06)	0.03-0.20
Viola pallens	8	120 (200)	5-600	0.54 (0.37)	0.04-1.13

Table 7

Concentrations of Arsenic and Mercury in shoots and roots of plants associated with Oldham tailings

Plant Species	Arsenic (ppm)		Mercury (ppm)	
	Shoots	Roots	Shoots	Roots
Grass				
Leersia oryzoides	300	2700	0.2	1.6
Sedges				
Dulichium grundinaceum	400	3100	0.2	0.7
Rushes				
Juncus pelocarpus	300	5600	0.5	6.1
Juncus articulatus	600	6300	0.6	2.7
Horsetails				
Equisetum fluviatile	700	3100	0.5	0.5
E. fluviatile (uncontaminated)*	2	-	-	-
Filamentous Green Algae				
Microspora quadrata	2800	-	3.2	-
M. quadrata (uncontaminated)*	13	-	-	-
Bur-reeds				
Sparganium fluctuans	4300	-	16.3	-

* from Dale and Freedman, 1982

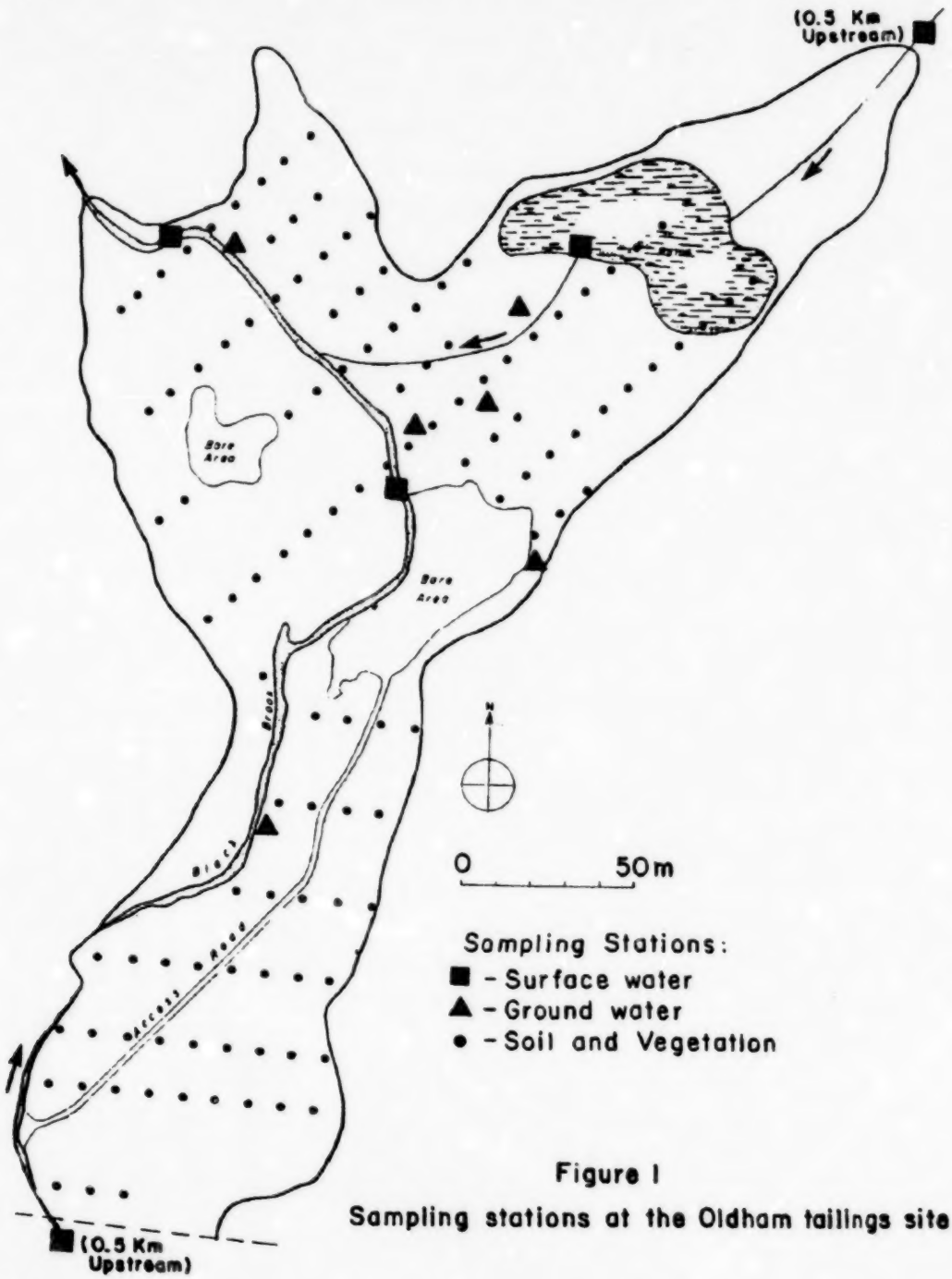


Figure 1

Sampling stations at the Oldham tailings site

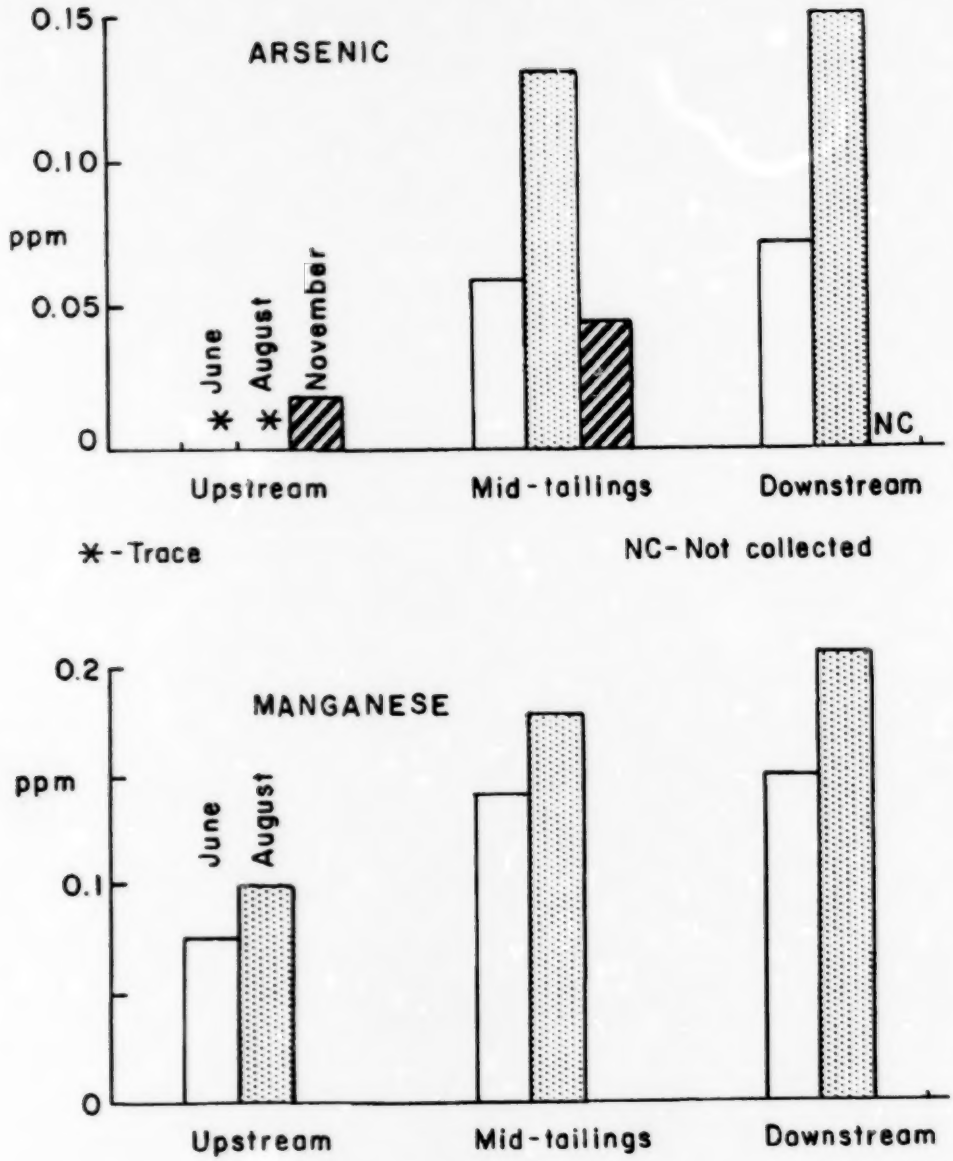
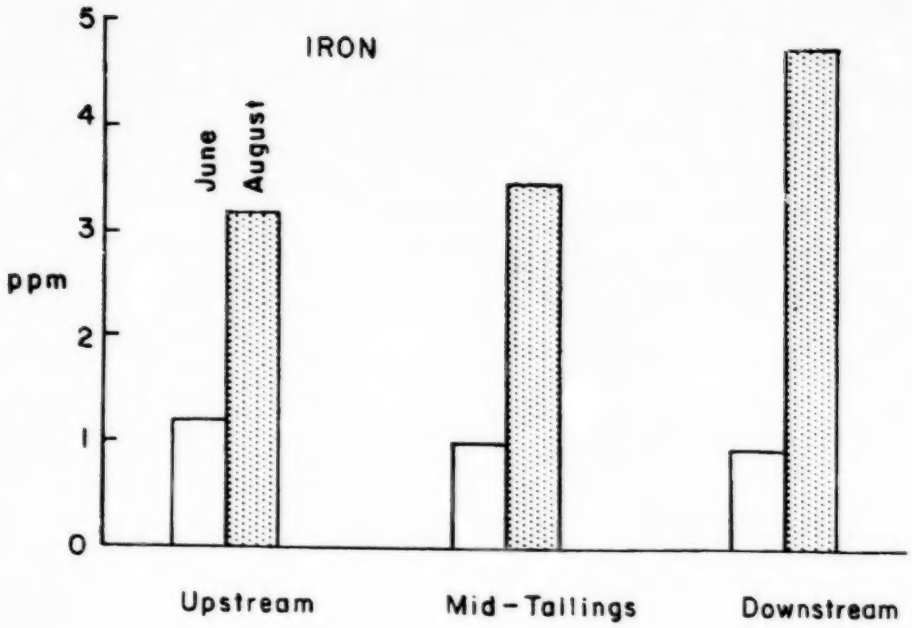


Figure 2

Arsenic and Manganese concentrations in surface waters taken at three stations in Black Brook during June, August and November, 1988



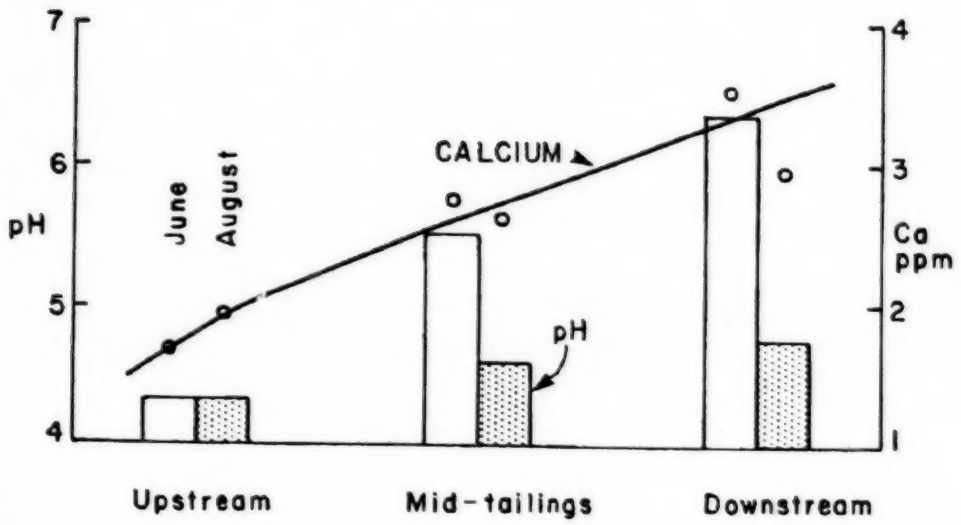


Figure 3

Iron and Calcium concentrations and pH of surface waters at three stations in Black Brook during June and August, 1988

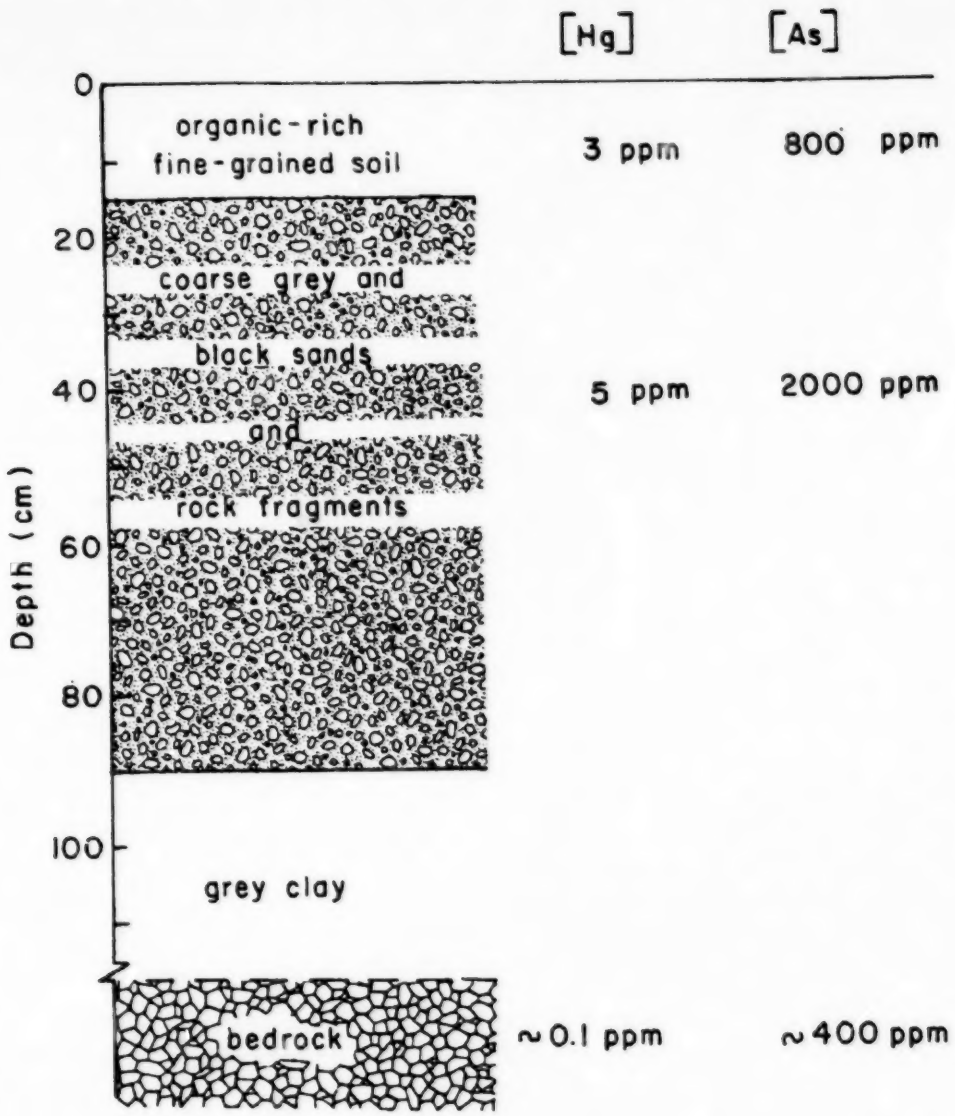


Figure 4

Total Arsenic (As) and Mercury (Hg) concentrations in a typical soil profile at the Oldham tailings site

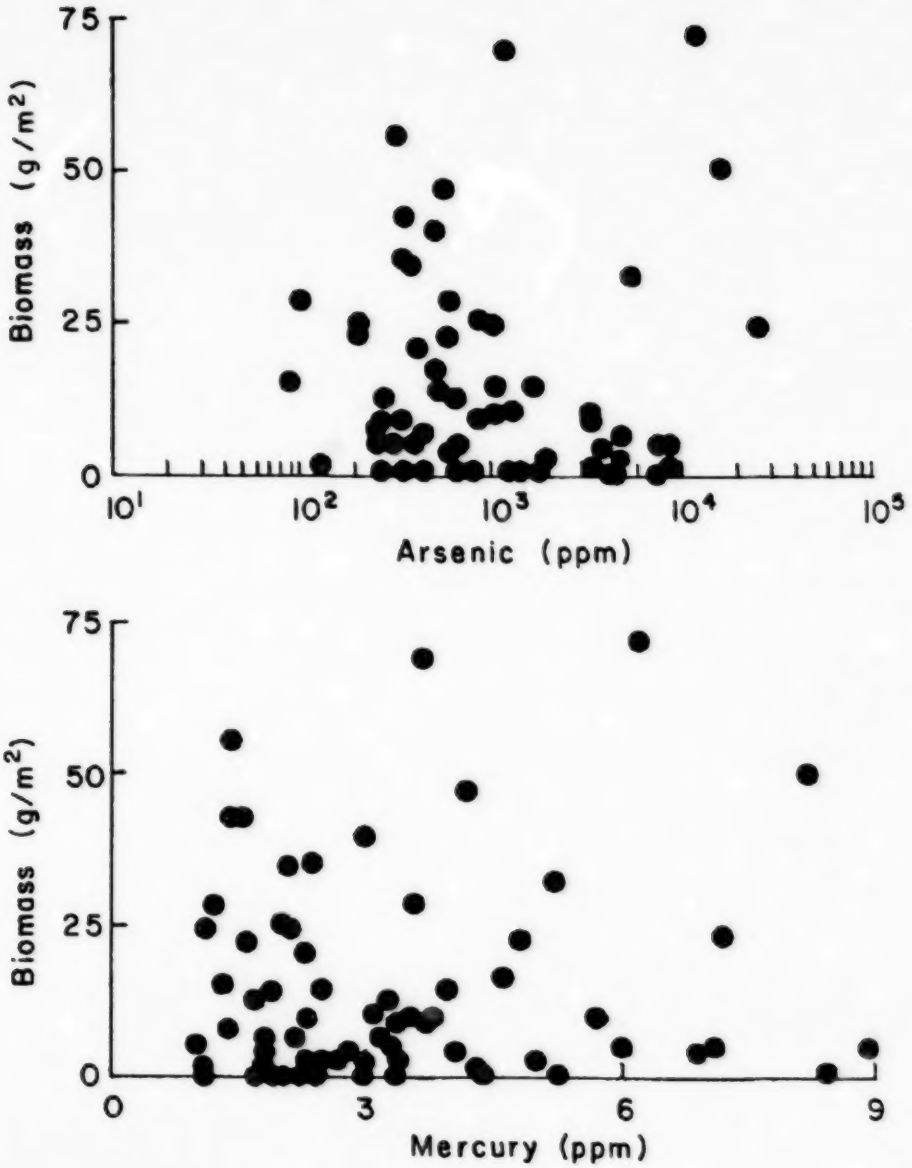


Figure 5

Biomass of a grass species, Agrostis palustris, growing in soils contaminanted with Arsenic and Mercury

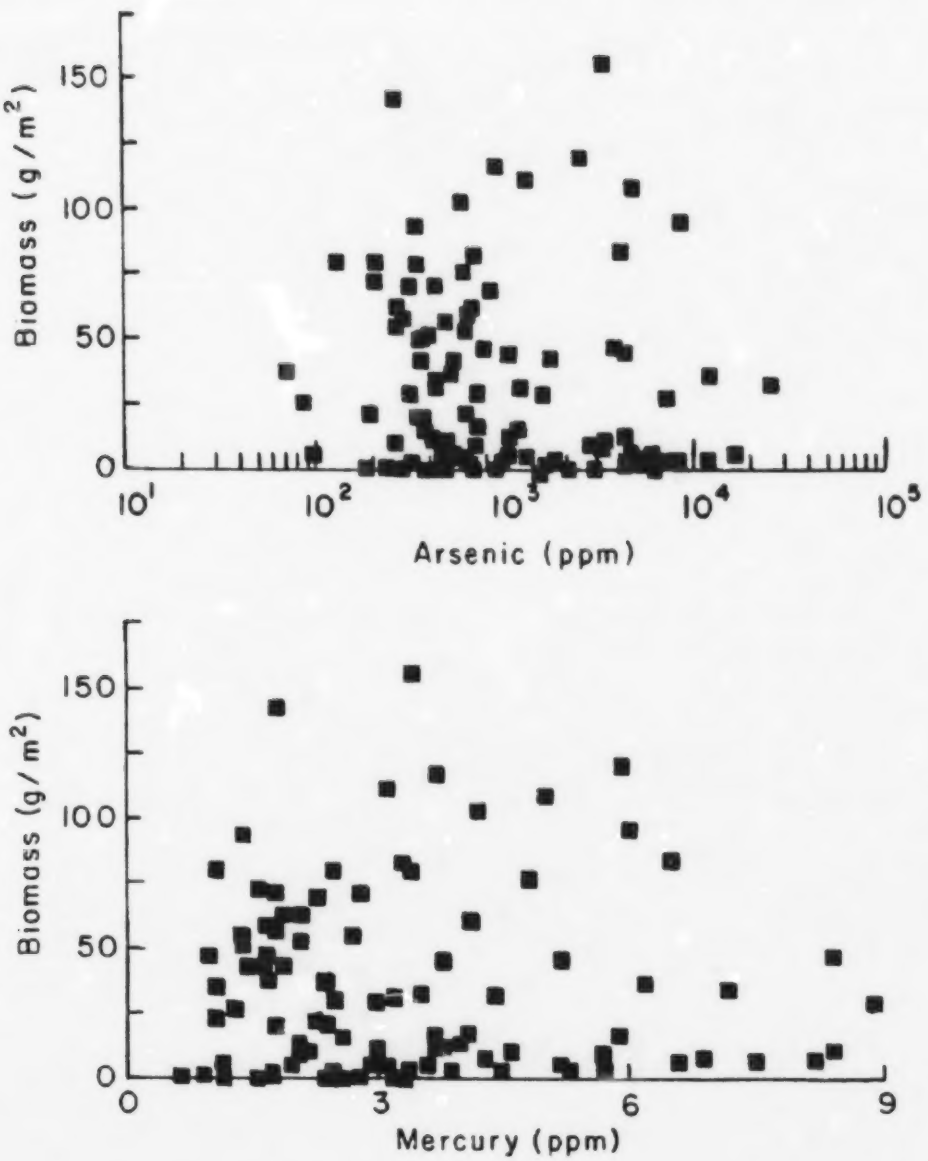


Figure 6

Biomass of a horsetail species, Equisetum fluviatile, growing in soils contaminated with Arsenic and Mercury

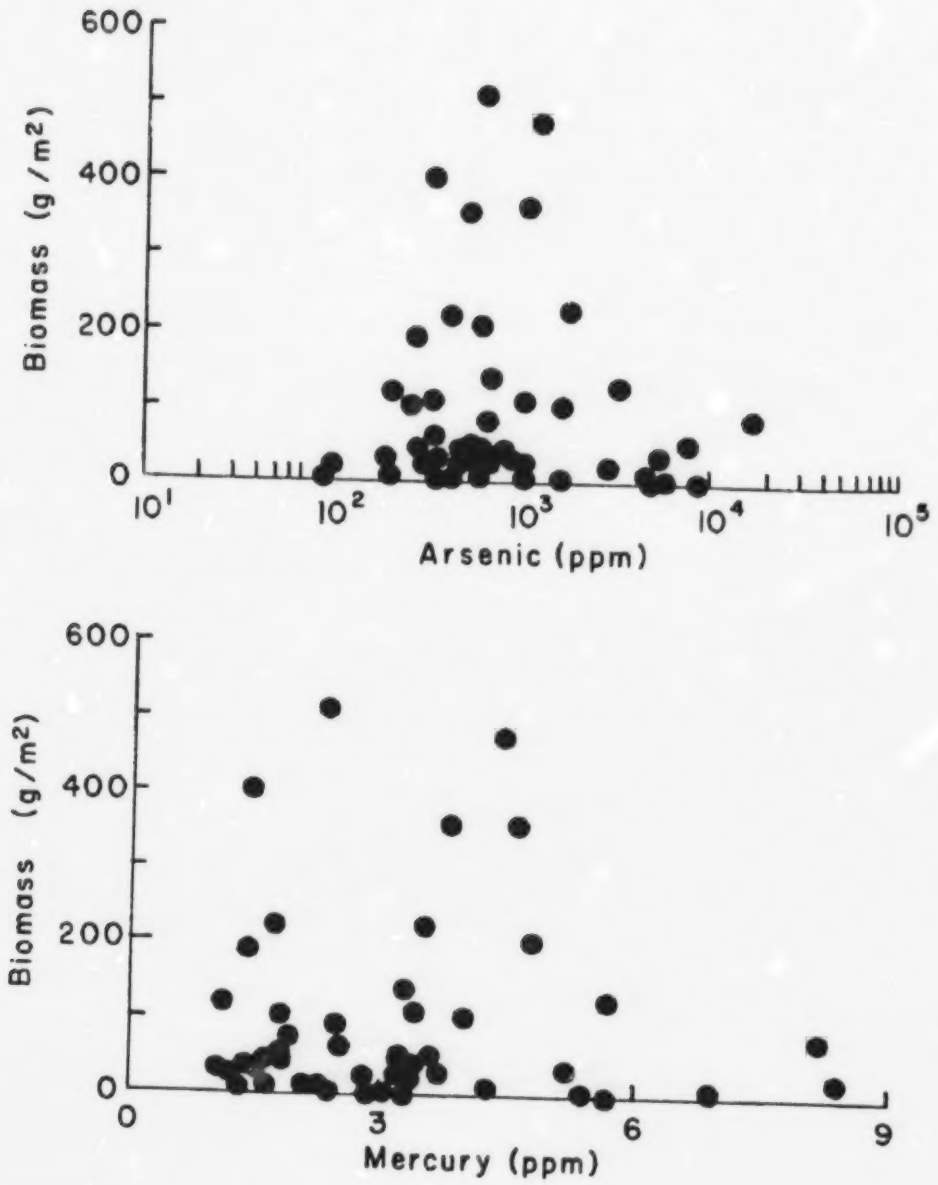


Figure 7

Biomass of a moss species, Campylium stellata, growing on soil contaminated with Arsenic and Mercury

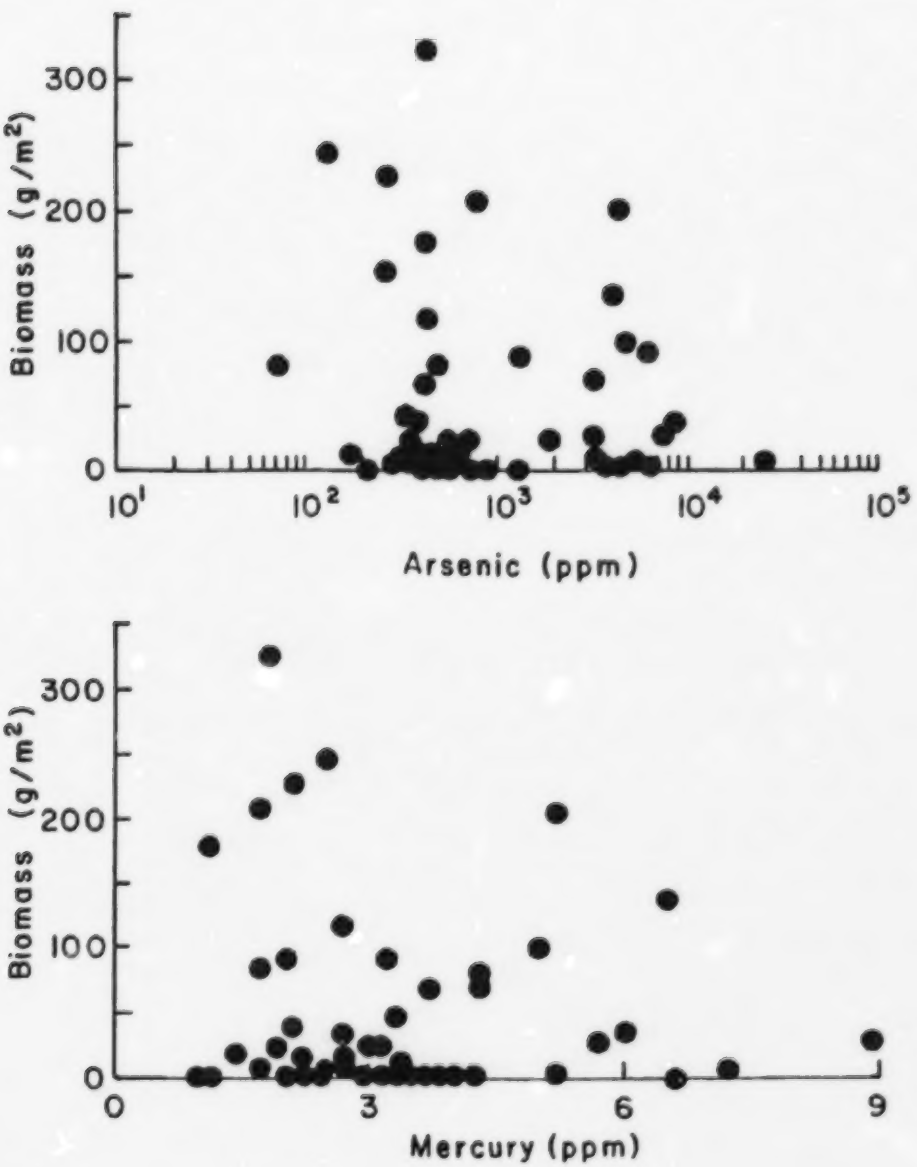


Figure 8

Biomass of a rush species, Juncus articulatus, growing in soils contaminated with Arsenic and Mercury

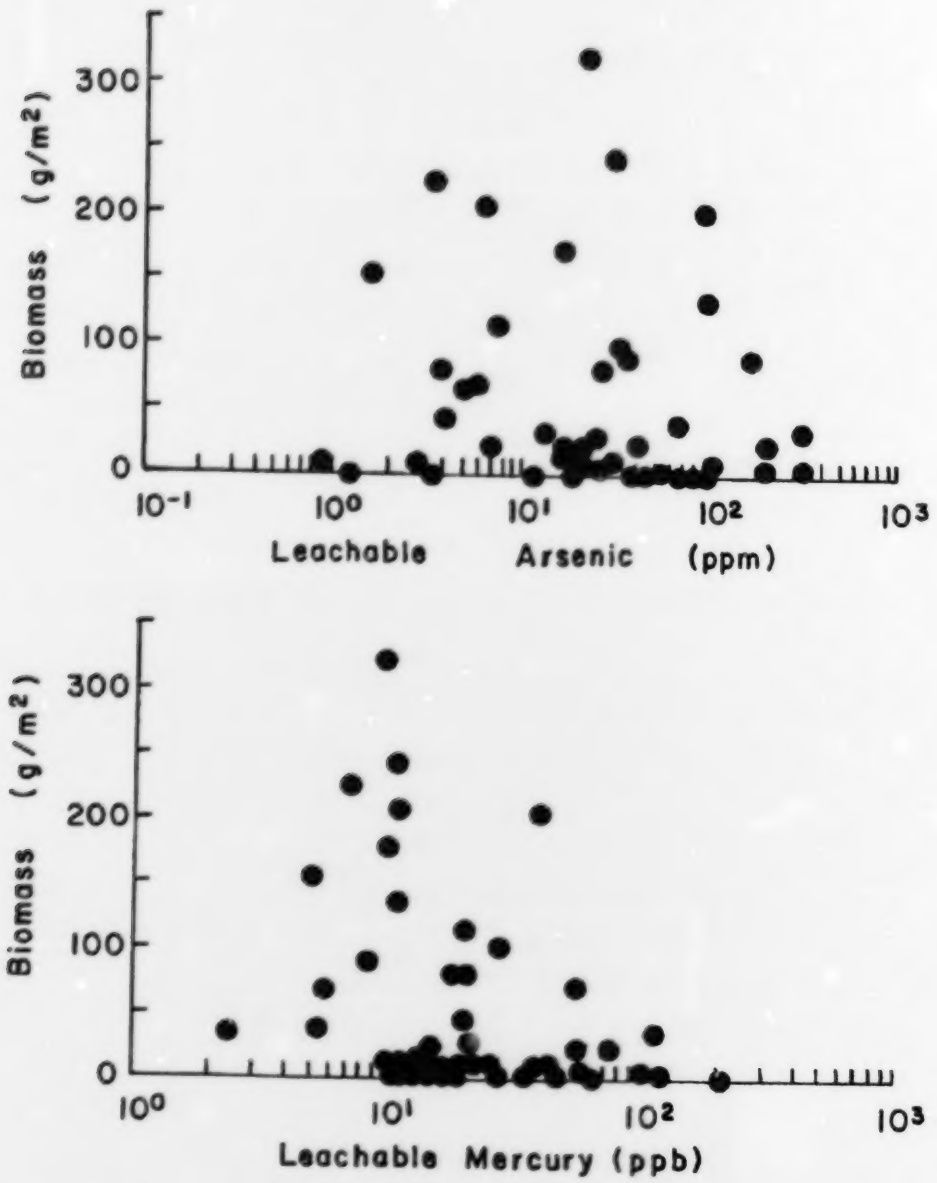


Figure 9

Biomass of Juncus articulatus.
plotted against leachable concentrations of Arsenic and Mercury

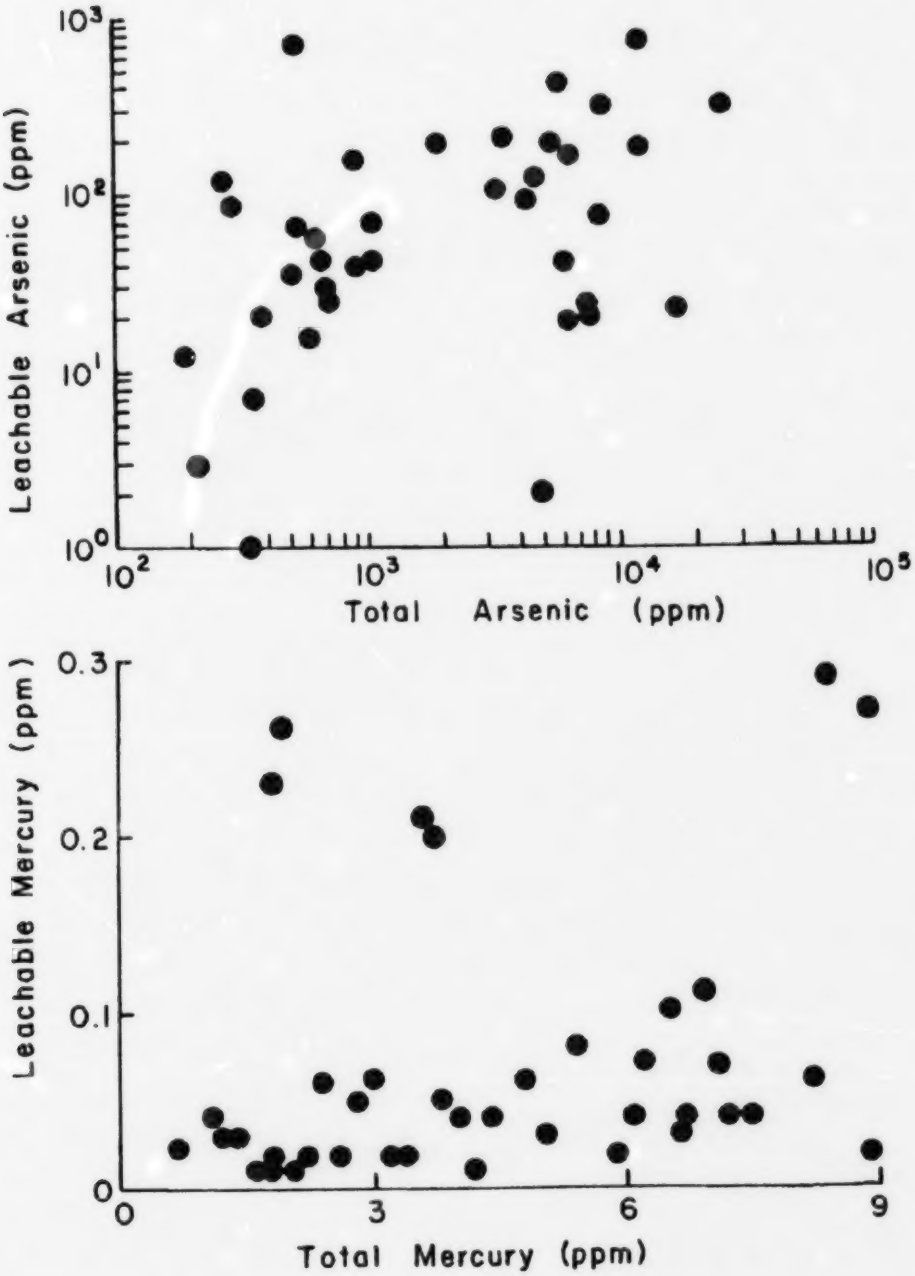


Figure 10

Leachable and total concentrations of
Arsenic and Mercury in surface soil samples

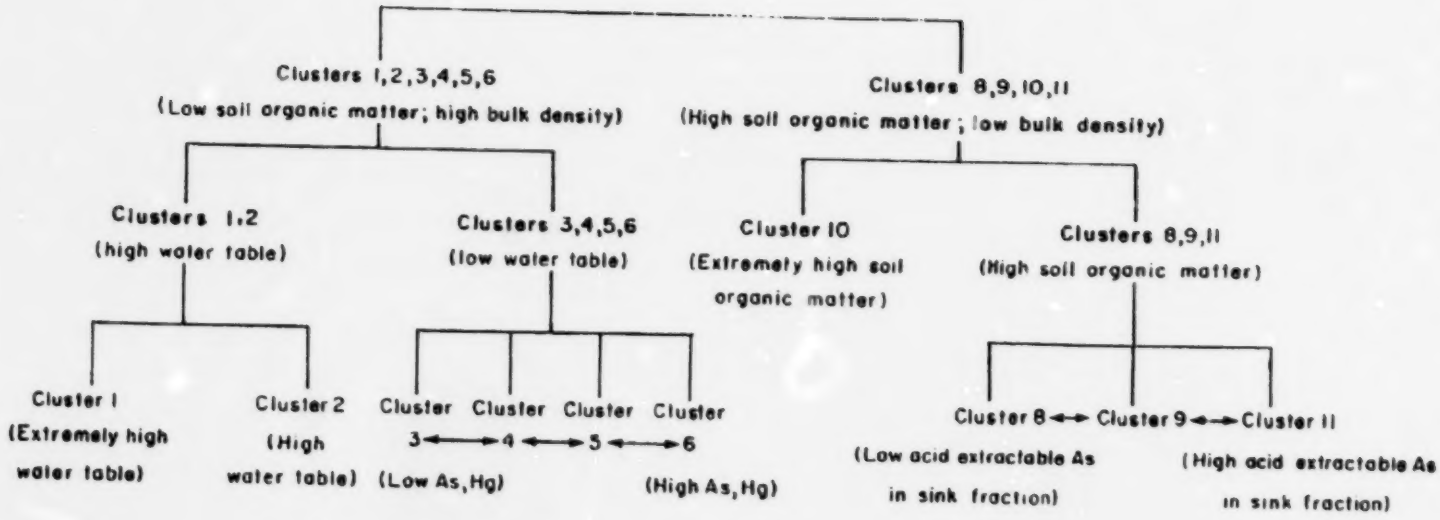


Figure 11

Dendrogram separating plant communities based on significant differences between physical and chemical features of the surface soils

The Development of an Economical Growth Medium for the Production of a Uranium-Adsorbing Fungal Biomass

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Abstract

Biotechnology is being increasingly used in the beneficiation of ores and waste material in the mining industry. Uranium removal from bacterially leached mine waters has been studied under contract to CANMET by ORTECH International as well as other researchers. However, because the metal values are often quite low in mine waters, current methods are inadequate to address the metallurgical and engineering problems to justify any recovery. Costs must also be minimized in the use of this form of biohydrometallurgy. The development of an inexpensive growth medium for the uranium-adsorbing fungus *Rhizopus arrhizus* is important in the production of large quantities of biomass. Fourteen industrial-based food processing wastes were obtained from various sources in southern Ontario. Carbon, nitrogen and phosphorous were determined for each sample and then prepared to concentration, which would theoretically maximize growth to 20 to 25 g biomass per litre of medium. Screening tests resulted in brewery grain-based waste media being selected for further growth optimization.

Résumé

La biotechnologie est de plus en plus utilisée pour enrichir les minerais et les déchets miniers. L'extraction de l'uranium des eaux de lixiviation bactérienne a fait l'objet d'études à la suite de contrats octroyés par CANMET à des groupes de chercheurs, notamment à ORTECH International. Cependant, comme la teneur en métal des eaux minières est souvent très faible, les méthodes actuelles ne permettent pas de résoudre les problèmes métallurgiques et techniques et de justifier ainsi toute récupération. Il faut en outre minimiser les coûts découlant de cette forme de biohydrométallurgie. La mise au point d'un milieu de croissance peu coûteux pour le champignon d'adsorbtion de l'uranium *Rhizopus arrhizus* est important pour la production de grandes quantités de biomasse. On a analysé 14 échantillons de déchets alimentaires industriels de diverses sources réparties dans le sud de l'Ontario. On a déterminé la teneur en carbone, en azote et en phosphore de chaque échantillon avant concentration, ce qui devait en maximiser théoriquement la croissance de la biomasse jusqu'à une quantité variant entre 20 et 25 g par litre de milieu. Après des essais de tamisage, on a choisi un milieu de résidus de brasserie que l'on a utilisé pour optimiser la croissance de la biomasse.

Introduction

Industrial waste as a growth medium is considered economically suited to maximizing developing biotechnology. The waste materials are largely agricultural commodity products, so generally the price is such that they can be used in commercial production. Most of the products are also available in large enough quantities that supply should not be a problem in large-scale biomass production.

The Department of Supply and Services awarded a contract (DSS 23440-8-9198/01-52) to ORTECH International to investigate the feasibility of using industrial food waste as a source of growth medium for the uranium adsorbing fungus *R. arrhizus*. The study looked at key characteristics of the biomass such as uranium-adsorption values and downstream processing considerations.

Background

Various microorganisms have been documented as having the unique characteristic of being able to concentrate metals from aqueous solutions. The ability of fungi to survive in the extremely acidic (pH 2) underground leach waters of the uranium mines of Denison Mines Limited (Elliot Lake, Ontario) has resulted in the isolation of a *Penicillium* sp. fungus with a high uranium-adsorptive capacity. However, variability in the growth of the organism has resulted in less than optimal biomass production. Uranium has also been successfully extracted from the uranium-laden mine waters using the fungus *R. arrhizus*. Optimal growth conditions have been established using a proprietary nutrient-glucose medium, but not in a cost-efficient medium. The abundance of various industrial wastes is seen as a viable alternative to costly media preparations in the production of biomass for uranium-extraction.

Non-viable *R. arrhizus* biomass has been shown to have the capacity to uptake 180 mgU/g dry weight biomass (M. Tzesos, B. Volesky, Biotechnology BioEngineering 23, 583, 1981), more than double the capacity of IRA-400, a common ion-exchange resin used for the accumulation of uranium. It therefore has the potential for incorporation into a hydrometallurgical processing scheme. To keep this project applicable to proven metal extraction processes, the focus of this paper will be to consistently produce 20 to 25 g wet weight biomass per litre food waste medium capable of uranium-adsorption values of 150 to 200 mgU/g dry weight biomass and with hydrodynamic characteristics amenable to ion exchange concepts.

Development

The research program consisted of three main stages:

- survey of industrial food producers to determine available wastes;
- optimization of growth medium and biomass production; and
- testing of uranium loading and elution.

Materials and Methods

After the waste products were obtained, total organic carbon (TOC), total kjeldahl nitrogen (TKN) and total phosphorous (TP) were chemically analyzed.

Food waste media were initially prepared using autoclaved, raw Oster-blended samples. The samples were prepared using the above chemical analysis and the following assumptions as guidelines:

- 20 to 25 g wet weight biomass per litre of medium (4 to 5 g dry weight);
- 0.4 to 0.5 g dry weight cells per g glucose;
- ≤ 13% nitrogen and ≤ 3% phosphorus in cellular composition; and
- essential and trace elements incorporated in dilution tap water.

Because glucose was probably not part of the food waste, excess waste was incorporated into 100 mL samples for screening test assays. Visual assessments were made on the fungal growth in these preparations with further development taking place using extracts of the raw food waste showing good growth characteristics.

Extracts of raw food waste were prepared using measured quantities of waste in 1 L volumes of tap water. The water was boiled with the waste, then muslin-filtered, frozen, filtered again through #1 Whatman paper, then autoclaved in 100 or 200 mL aliquots. Wastes were used in quantities that would allow for theoretically maximum values of TOC, incorporated in double or more quantities than required for 20 to 25 g biomass/L medium. Specific gravity (SG) measurements were made to approximate soluble carbohydrate values, as compared with the SG of known sucrose solutions.

Growth of *R. arrhizus* was followed with gravimetric determinations of biomass harvested from a series of five or six flasks over 40 to 75 h. After gravimetric assessments of biomass yields grown in the extracts were prepared, only two wastes were kept for further optimization: spent brew grain (SBG) and cereal. Growth curves were prepared for repeated experiments with the SBG and cereal extracts, with SBG being selected for final optimization.

An optimal inoculum concentration was determined by using either 1 mL of a 2.5×10^5 or 2.5×10^6 spores per millilitre spore suspension, and by monitoring growth characteristics both morphologically and gravimetrically.

Medium strength and agitation rates were also studied. Full strength (FS) extract (200 g/L) and double strength (DS) extract (400 g/L) were agitated at two different gyrotary shaker speeds: 200 rpm and 300 rpm. Growth curves of these experiments were prepared from gravimetric determinations.

The fungus was grown in 200 mL volumes to obtain enough for bioadsorption assays. Up to nine flasks would be shaken as described, harvested on three layers of cheesecloth, washed in warm water for 30 min and then freeze-dried. The biomass would be harvested before the characteristic rise in pH associated with the death phase of the culture.

Uranium adsorption tests were conducted at only one solid-to-liquid ratio, that is 1:200. As-received Denison Mines water (pH 2.45) was adjusted to pH 4 and filtered through a 0.45 μm Millipore unit before bioadsorption tests. Aliquots of 100 mL mine water were contacted with 0.5 g of two different biomasses:

DS 200 rpm and FS 300 rpm. The tests were conducted over 1 h, with samples taken at 5 min, 15 min, 30 min and 60 min. These samples were filtered using a 0.45 μm Millipore unit and then submitted for uranium analysis by DCP.

Results and Discussion

A survey of 30 food producers in Southern Ontario (see Table 1) resulted in an agreement among eight to supply 14 different food wastes (see Table 2). Of the 30 producers, 10 produced no waste; 5 had seasonal waste and it was not available at the time of the investigation; 2 had bureaucratic complexities; 2 had undesirable waste; 1 was too far away; 1 recycled its waste; and 1 landfilled its waste.

Chemical analysis for TKN, TOC and TP (see Table 3) revealed very low TKN values for onions and chicken waste water, which concluded any further investigations with these wastes. Because 4 to 5 g dry weight biomass per litre of medium was the goal of this study, both the above wastes would have needed nitrogen supplementation to provide the 10 to 13% cellular nitrogen requirements.

Initial screening tests for growth were conducted using raw, blended food-waste preparations. Because of the large percentage of insoluble, non-glucose type carbohydrate, excess food waste was used in each preparation to increase the potential for the presence of metabolizable carbon. At the same time, phospherus and nitrogen would have been potentially present in non-limiting concentrations.

Biomass determinations were unfeasible because of the non-separable, fibrous nature of the blended wastes. Therefore, visual assessments of the mycelial growth habit of *R. arrhizus* were used to select wastes with biomass harvest potential. No growth was observed in the vitamin beverage or pig slop, while only very slight growth accompanied the mushroom compost and the mixtures of food wastes. Good to moderate growth occurred with the other eight wastes: bean dirt, cereal, fish, grain screenings, potato chips, corn and SBG.

Boiling water extracts were made of these eight food wastes, resulting in growth media that were clear and homogeneous, although chemically undefined. Nitrogen, carbon and phosphorous values had to be implied from the amount of food waste used in the preparation, as well as the measured SG. SG measurements allowed for a comparison to known sucrose solutions and are illustrated by the following examples.

1. SBG
   23 g TOC/200 g
   SG 1.002 = 10 g sucrose/L

2. cereal
   20 g TOC/50 g
   SG 1.003 = 15 g sucrose/L

Although sucrose is unlikely to be present as a carbohydrate source, the SG of these extracts does give an idea of how much soluble carbohydrate may be present. Maximum SG measurements ranged from 1.007 to 1.002 and were, in descending order: potato chips, grain screenings, fish, cereal, corn, bean dirt and SBG.

Maximum biomass yields were stable in the cereal and SBG media and were poor in the potato and corn media. Possible explanations for poor results could include the absence of growth factors, trace nutrients or amino acids; the presence of growth inhibitors such as the salt in chips; or the availability of nitrogen to the organism. Based on these results, the cereal and SBG were chosen for more investigation.

Preparing the cereal extract was difficult because of filtration problems, but SBG presented no such problems. Growth experiments in either medium allowed for further reduction of the number of wastes to just one. Figure 1 shows the cereal extract producing a slightly faster initial growth rate while the SBG extract produces more biomass per litre at about 60 h (2.0 g dry weight compared with 1.5 g dry weight). These results along with repetitive similar assays, allowed for the selection of SBG for further optimization.

The optimal spore concentration to use as inoculum was determined as 1 mL of a 2.5×10^5 spores/per millilitre suspension. A higher spore concentration, 2.5×10^6 spores per millilitre, resulted in poor to no mycelial development with only slight development of the inoculum into the "pellet" growth habit.

Agitation speed and medium strength were investigated in the SBG extract optimization work. Agitation at 200 rpm (see Figure 2) of both FS and DS SBG extract resulted in significantly higher biomass yields in the DS medium at 40 h (2.5 g dry weight compared with 1.2 g dry weight). Considerably more biomass was produced when the DS medium was agitated at 300 rpm (4 to 5 g dry weight per litre compared with $\leq$ 0.6 g dry weight per litre) (see Figure 3).

Uranium adsorption by the harvested and processed biomass proceeded with two different biomasses: the fungus grown by the FS medium agitated at 300 rpm (FS 300, see Figure 5) and the DS medium agitated at 200 rpm (DS 200, see Figure 4). Using a sample of Denison Mines water with about 130 ppm uranium in solution at pH 4, and a solid-to-liquid ratio of 1:200 (0.5 g biomass/100 mL mine water), at least 95% of the uranium was removed within 5 min by both biomasses.

Although most of the uranium was removed from solution, a biosorption rate of 25 mgU/g biomass was attained, not the 200 mgU/g as proposed. By altering the solid-to-liquid ratio, ideal results may be attainable. Not only was nearly all the uranium removed from solution, but the hydrodynamic properties of the biomass were also positive. The absence of any floating biomass meant that the biomass is capable of being developed into an ion-exchange system.

Summary

From biomass gravimetric determinations, it has been found that 20 to 25 g wet biomass per litre can be grown using an extract prepared from SBG. An extract made from 400 g SBG/L and cultured at 300 rpm on a gyrotary shaker have been determined as optimal growth conditions.

Maximum biomass uranium-loadings have been achieved for the solid-to-liquid ratio used. Therefore, the potential exists for increased uranium-loadings to 200 mg/g biomass.

The freeze-dried biomass did not float, indicating hydrodynamic properties amenable to existing extracting technologies.

Conclusion

Biomasses such as these are unlikely to replace conventional uranium-concentration or purification processes such as IX or SX. However, where low tenor solutions are present, these biomasses have the distinct advantage of low costs and low maintenance.

With one-time applications or reusability potential of the biomass technology, there are certain hydrometallurgical situations that might benefit. For example:

- acid tailings run-off, where downstream removal of metals could help prevent toxic metal contaminations of our environment: and
- underground bioleach solutions, where column contacting may be incorporated, as well as the advantage of recycling existing leach water.

The key factor in these situations is the use of an inexpensive biomass. It is a process option.

Table 1
Survey of Industrial Food Producers
and the Waste Produced
in Southern Ontario

Producers	Result
Freshway Products	no waste available
McCarthy Milling	no waste available
Connors Brewery	spent brew grain available
St. Lawrence Corn Starch	all waste to alcohol production
Imperial Flavors Inc.	no waste
Gay Lea (Yogurt)	no waste
Campbell's Mushroom Farm	hay, mushrooms, compost
Campbell's (Chatham)	seasonal waste
Canada Packers Daily Div.	no waste available
General Mills	no waste available
Canada Malting	waste water in Thunder Bay
Reid Milling	no waste
Robin Hood Milling (Milton)	no waste
Maple Leaf Mills Rendering	no waste
Nabisco Foods	cereal rejects
Region of Peel	organic sludge matter
Ontario Food Terminal	variety, seasonal
E.D. Smith	seasonal waste
Heinz	seasonal waste
Omstead Foods (Wheatly)	fish, onions
Heritage Farms (Bramalea)	milk waste, pig slop
Hostess Foods (Cambridge)	chip waste, corn screenings, potato mash
Nestlé	no waste available
Region of Halton	landfilled
Village of Hensall	grain screenings
Amstel Brewery	no waste available
City of Toronto	no waste available
Protein Foods (Paris)	poultry waste
Victory Soya	soap stock
Pillsbury	corn husks, seasonal

Table 2

Industrial Food Producers Agreeing

to Supply Food Waste Products

Producer	Food Waste
Village of Hensall	1) bean dirt
	2) grain screenings
Omstead Foods	3) fish
	4) onions
Nabisco Foods	5) cereal rejects (Shredded Wheat and Shredd'es mixed)
Hostess Foods	6) potato mash
	7) plain potato chips
	8) corn mash
Heritage Farms	9) dairy vitamin beverage
	10) pig slop
Campbell's Mushroom Farm	11) mushrooms
	12) compost
Protein Foods	13) chicken waste
Connors Brewery	14) spent brew grain

Table 3

Nitrogen, Carbon and Phosphorous

Values of Food-Waste Samples

	TKN (ppm)		TOC (%)		Total P (ppm)	
	Original	After Dilution	Original	After Dilution	Original	After Dilution
Sample	Suspension	Calculation	Suspension	Calculation	Suspension	Calculation
Bean	4 830	19 320	7.81	31.24	712	2 848
Onion	570	1 140	1.12	2.24	167	334
Cereal	3 590	17 950	7.78	38.90	639	3 195
Fish	8 020	16 040	6.84	13.68	1 950	3 900
Grain	730	8 030	3.80	41.80	136	1 496
Potato mash	2 090	2 786	3.68	4.90	250	333
Potato chips	3 260	9 780	16.50	49.50	459	1 377
Corn	2 460	8 610	9.07	31.70	747	2 615
SBG	4 620	9 240	5.71	11.42	818	1 636
Mushroom	1 950	3 461	1.92	3.36	356	623
Mush. comp.	5 430	9 050	6.40	10.67	1 550	2 583
Vitamin bev.	5 200	5 200	15.00	15.00	423	423
Chicken w.w.	570	570	3.92	3.92	127	127
Bean	5 540	22 160	7.36	29.44	791	3 164
Onion	500	1 000	1.22	2.44	166	332
Pig slop	6 550	6 550	9.02	9.02	747	747

TKN: total kjeldahl nitrogen

TOC: total organic carbon

Total P: total phosphorous

SBG: spent brew grain

Table 4

Maximum Chemical Values of Extracts

	Food Waste								
	Spent Brew Grain		Cereal	Corn	Fish	Bean Dirt	Grain Screens	Potato Mash	Potato Chips
	FS	DS							
Amount per extract (g/L)	200	400	50	160	200	40	200	200	100
TOC* (g)	22.840	45.680	19.450	50.720	27.360	12.500	83.600	9.800	49.500
TKN* (g)	1.848	3.696	0.897	1.378	3.208	0.773	1.606	0.557	0.978
TP* (g)	0.372	0.744	0.160	0.418	0.780	0.114	0.299	0.066	0.137
SG	1.002	1.005	1.003	1.003	1.004	1.002	1.005	1.006	1.007
g sucrose/L **	10.0	18.0	12.5	12.5	15.0	10.0	17.8	20.1	23.0

* maximum values in the raw food waste

** for comparison only

TOC: total organic carbon

TKN: total kjeldahl nitrogen

TP: total phosphorous

SG: specific gravity (tap water = 1.000)

FS: full strength

DS: double strength

Figure 1
Comparison of Growth in Cereal and Spent Brew Grain

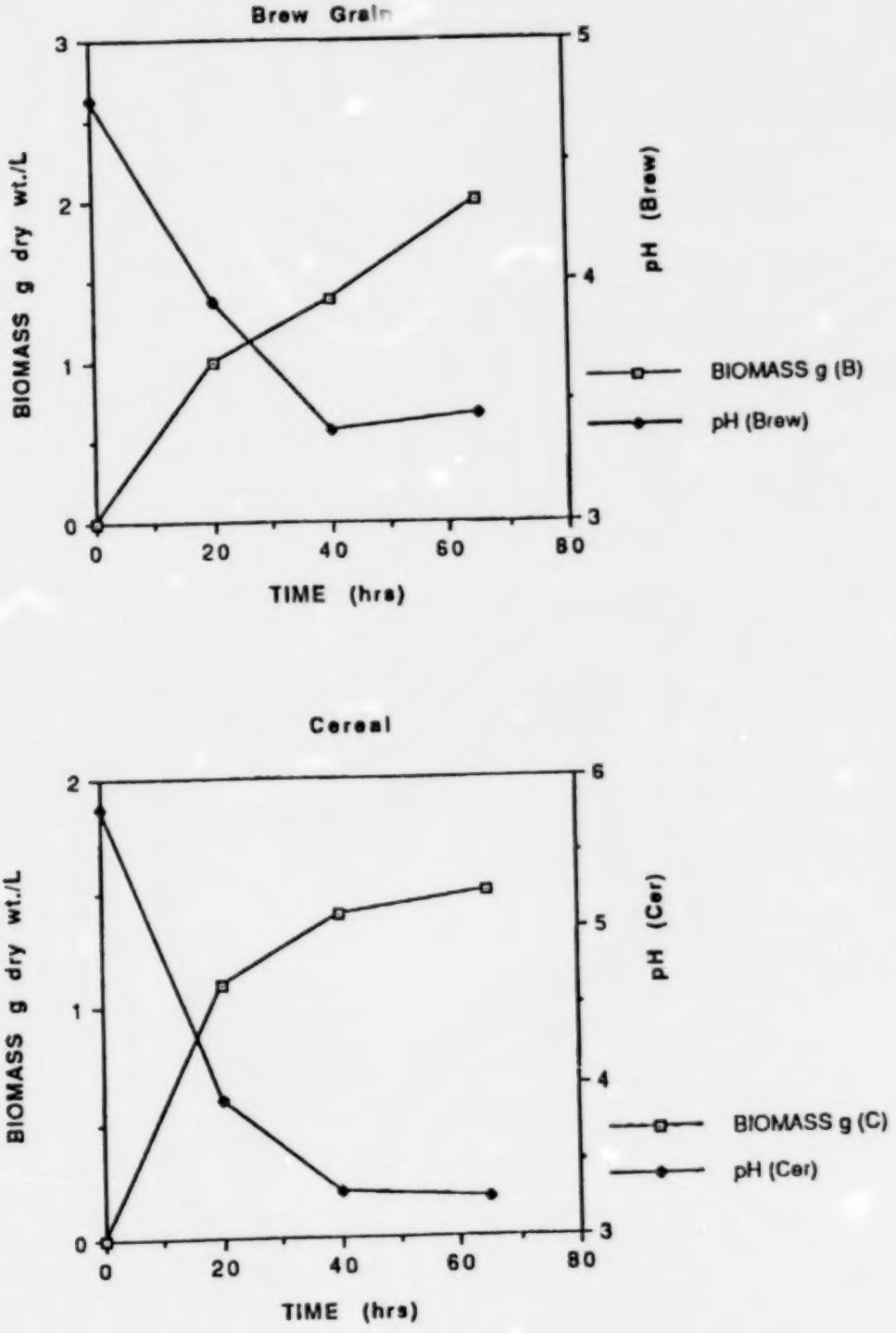

Figure 2
Comparison of Growth of *R. arrhizus* in Full Strength and Double Strength Agitated at 200 rpm

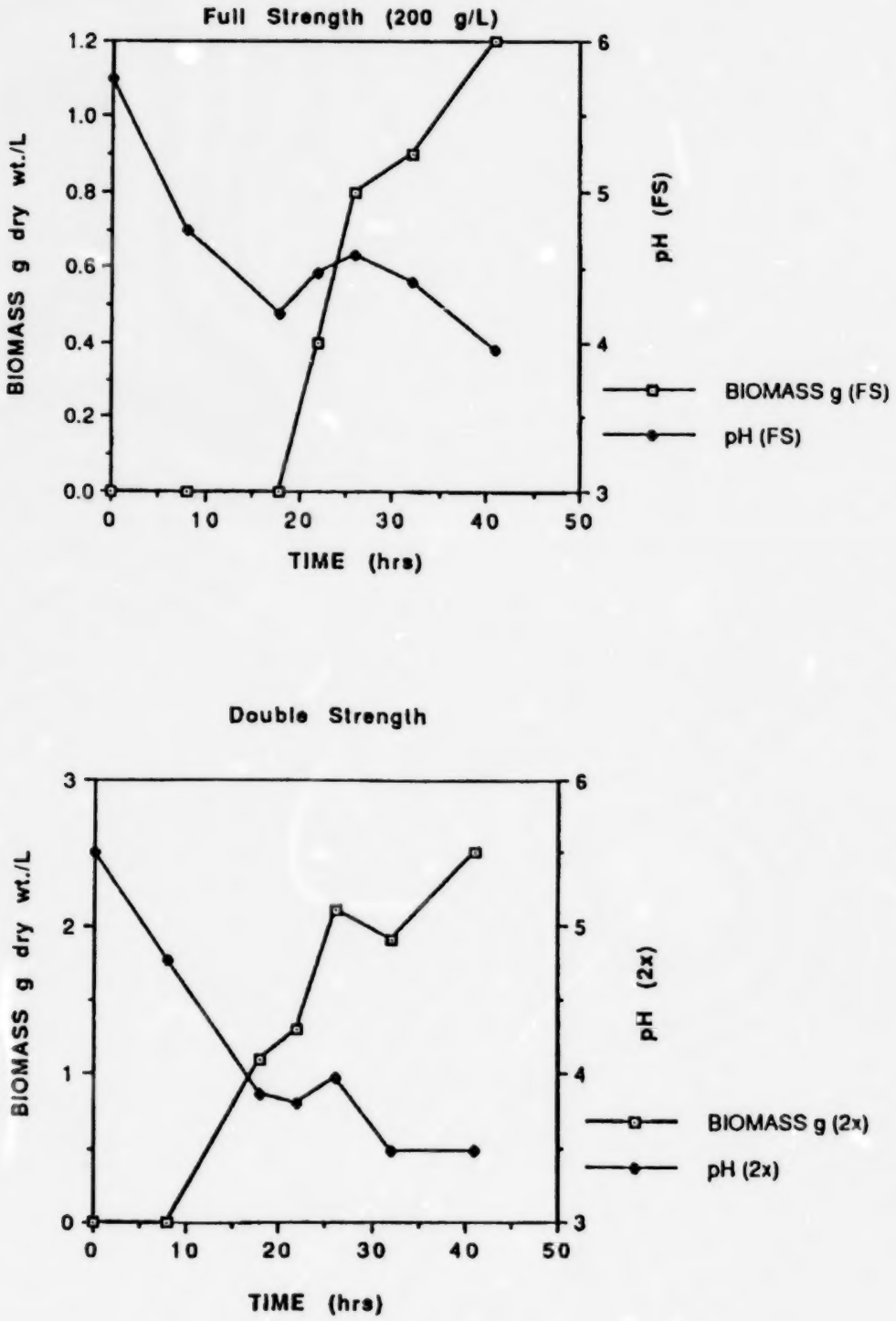

Figure 3
Comparison of Growth of *R. arrhizus* in Full Strength and Double Strength Agitated at 300 rpm

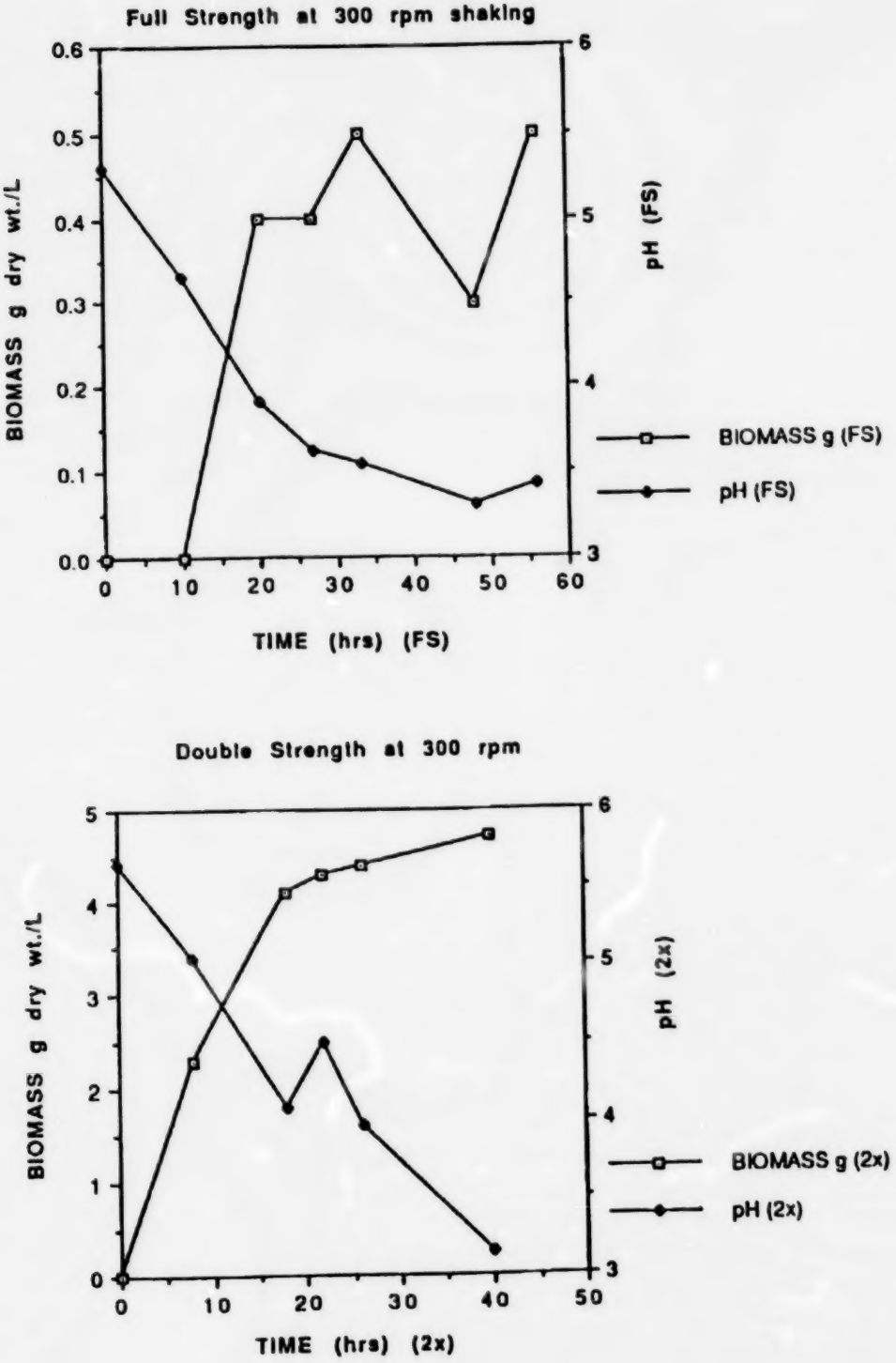

Figure 4
Removal of Uranium from Denison Mines Water by *R. arrhizus* Biomass (DS 200) in a Solid-to-Liquid Ratio 1:200

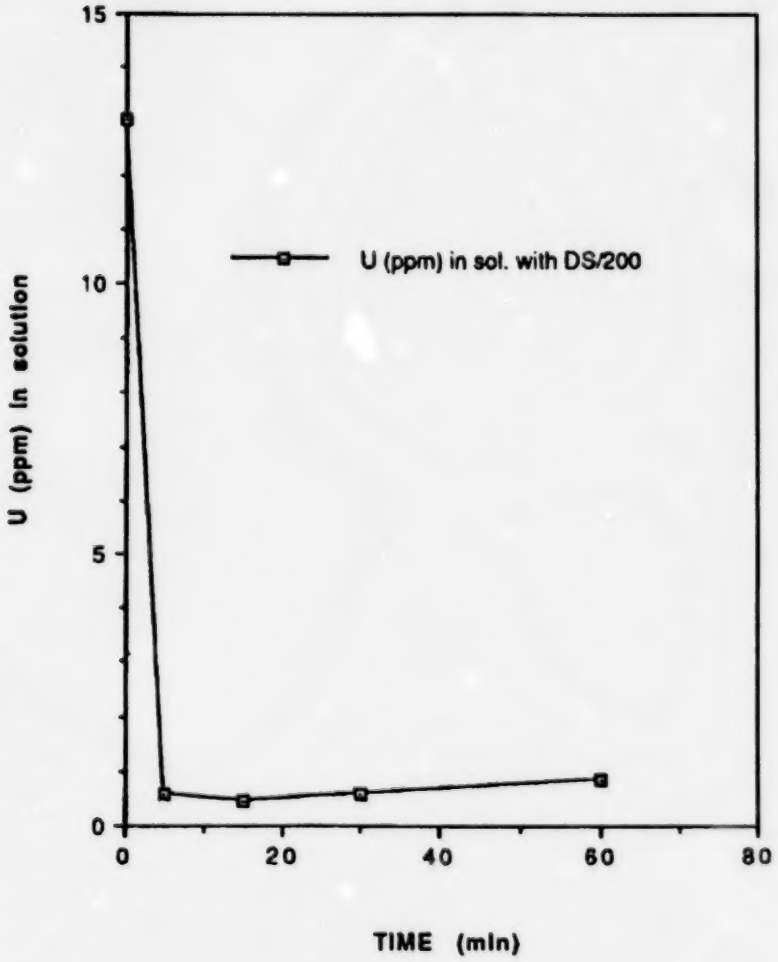

Figure 5
Removal of Uranium from Denison Mines Water by *R. arrhizus* Biomass (FS 300) in a Solid-to-Liquid Ratio 1:200